



Vertzoni, Maria and Augustijns, Patrick and Grimm, Michael and Koziolk, Mirko and Lemmens, Glenn and Parrott, Neil and Pentafragka, Christina and Reppas, Christos and Rubbens, Jari and Van Den Abeele, Jens and Vanuytsel, Tim and Weitschies, Werner and Wilson, Clive G. (2019) Impact of regional differences along the gastrointestinal tract of healthy adults on oral drug absorption : an UNGAP review. European Journal of Pharmaceutical Sciences, 134. pp. 153-175. ISSN 0928-0987 , <http://dx.doi.org/10.1016/j.ejps.2019.04.013>

This version is available at <https://strathprints.strath.ac.uk/67946/>

Strathprints is designed to allow users to access the research output of the University of Strathclyde. Unless otherwise explicitly stated on the manuscript, Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Please check the manuscript for details of any other licences that may have been applied. You may not engage in further distribution of the material for any profitmaking activities or any commercial gain. You may freely distribute both the url (<https://strathprints.strath.ac.uk/>) and the content of this paper for research or private study, educational, or not-for-profit purposes without prior permission or charge.

Any correspondence concerning this service should be sent to the Strathprints administrator: strathprints@strath.ac.uk



Impact of regional differences along the gastrointestinal tract of healthy adults on oral drug absorption: An UNGAP review

Maria Vertzoni^{a,*}, Patrick Augustijns^b, Michael Grimm^c, Mirko Koziolk^c, Glenn Lemmens^b, Neil Parrott^d, Christina Pentafragka^a, Christos Reppas^a, Jari Rubbens^b, Jens Van Den Abeele^b, Tim Vanuytsel^e, Werner Weitschies^c, Clive G. Wilson^{f,*}

^a Department of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Greece

^b Drug Delivery and Disposition, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Belgium

^c Department of Biopharmaceutics and Pharmaceutical Technology, Center of Drug Absorption and Transport, University of Greifswald, Germany.

^d Pharmaceutical Sciences, Pharmaceutical Research and Early Development, Roche Innovation Centre Basel, F. Hoffmann-La Roche Ltd., Basel, Switzerland

^e Translational Research Centre for Gastrointestinal Disorders (TARGID), Department of Chronic Diseases, Metabolism and Ageing (ChroMeTa), KU Leuven, Belgium

^f Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), University of Strathclyde, UK

ARTICLE INFO

Keywords:

Oral drug absorption
Fasted state
Fed state
Luminal environment
Unstirred water layer
Imaging techniques

ABSTRACT

Oral administration is the most common route of drug delivery. The absorption of a drug from the gut into the bloodstream involves disintegration of the solid dosage form, dissolution of the active pharmaceutical ingredient and its transport across the gut wall. The efficiency of these processes is determined by highly complex and dynamic interplay between the gastrointestinal tract, the dosage form and the API. The European Network on Understanding Gastrointestinal Absorption-related Processes (UNGAP) aims to improve our understanding of intestinal drug absorption by creating a multidisciplinary Network of researchers from academia and industry engaging in scientific discussions. As part of the basis for the UNGAP project, this review aims to summarize the current knowledge on anatomy and physiology of the human gastrointestinal tract with emphasis on human studies for the evaluation of the regional drug absorption and the prediction of oral dosage form performance. A range of factors and methods will be considered, including imaging methods, intraluminal sampling and, models for predicting segmental/regional absorption. In addition, *in vitro* and *in silico* methods to evaluate regional drug absorption will be discussed. This will provide the basis for further work on improving predictions for the *in vivo* behavior of drug products in the gastrointestinal tract.

1. Introduction

The modern diet is extremely rich, well balanced in terms of diversity and energy dense. This would have represented a rare occurrence to our foraging and hunting ancestors – a banquet at every meal, and as a consequence our bodies still function to make the most of the occasion. This is achieved through controlled exposure of the intestine to a preprocessed material and reduction by enzymes and motility to ever simpler building blocks to be catalyzed and placed into other metabolic pathways by the liver. Drugs, which are not nutrients (so-called ‘anutrients’ or ‘xenobiotics’) are similarly exposed to these processes. Regional specialization in anatomy and physiology is marked along the gut in terms of fluid composition, residence times, stirring and the nature of the epithelium which has consequences for drug absorption. Active pharmaceutical ingredients vary greatly in their

physicochemical properties: specifically solubilities at different pH and differences in permeability across the epithelia along the gut. Therefore presentation of drug at different points during transit will yield differences between formulations which are clinically important. In particular, the processing of food drives temporary changes in the environment which is why drug absorption has to be understood within a nutritional sciences context.

The gastrointestinal (GI) tract consists of a series of connected muscular tubes spanning from mouth to anus and its associated organs including the liver, gallbladder and pancreas (Fig. 1). The beginning of the digestion process is mastication. Chewing assists in an increasing surface area of the food matrix, aiding swallowing by shaping a lubricated bolus in the mouth. The oesophagus serves as a passage way for the food bolus between the mouth and the stomach and is delineated by the upper and the lower oesophageal sphincter. The lining of the:

* Corresponding authors.

E-mail addresses: vertzoni@pharm.uoa.gr (M. Vertzoni), c.g.wilson@strath.ac.uk (C.G. Wilson).

<https://doi.org/10.1016/j.ejps.2019.04.013>

Received 18 February 2019; Received in revised form 3 April 2019; Accepted 9 April 2019

Available online 13 April 2019

0928-0987/ © 2019 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

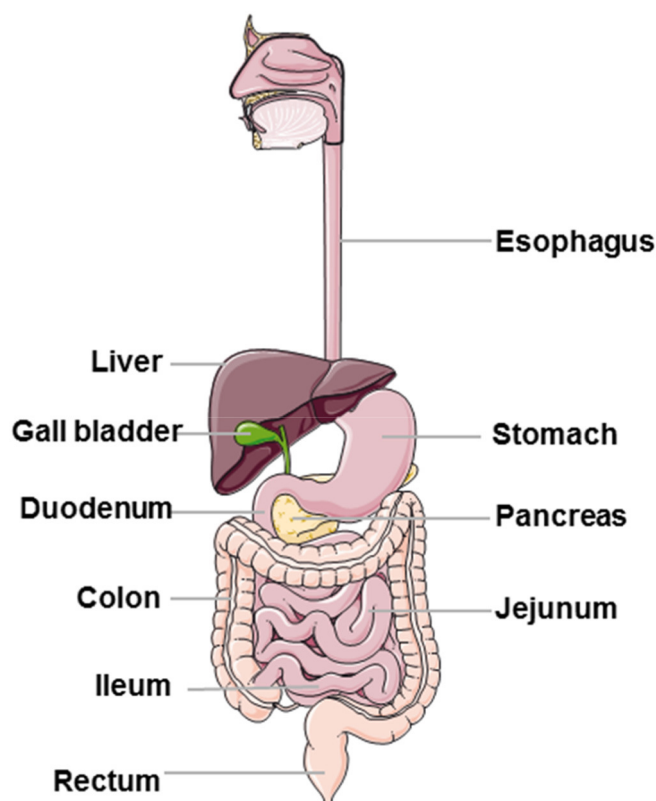


Fig. 1. The human gastrointestinal tract.

mouth and oesophagus, is composed of a surface layer stratified squamous epithelium which is a toughened, thickened layer and not useful for nutrient absorption.

Upon arrival of a bolus of food to the stomach, the proximal part of the stomach or *fundus* relaxes in a process termed ‘receptive relaxation’ or ‘accommodation’. While the fundus functions as a storage organ, the body or *corpus* and the distal part of the stomach or *antrum* are concerned with mixing the food with acid, pepsin and gastric lipase as a first step in the digestion process. The stomach has a low pH which reduces the bacterial bioburden and destroys some compounds that could be harmful. Emesis assists with the expulsion of contaminated food or poisons which could harm the body if entry to the systemic circulation occurs. Thus, the upper parts of the gastrointestinal tract do not have a role in direct nutrient absorption but serve to begin processing of the matrix to a more water dispersed form. The lining of these parts of the gut is squamous epithelium, transitioning to gastric secretory epithelium in the stomach. The stomach stores food as a wet solid/liquid and in combination with this the duodenum where an absorptive epithelium is encountered, begins to assess the nature and nutritive value of the meal. This allows emptying at the rate appropriate to the calorie content, allowing the other parts of the intestine, the jejunum and ileum, to process gastric chyme in an orderly controlled fashion. Contractions push the food forward against a closed sphincter, the *pylorus*, until the particles are small enough (2–5 mm) to pass through to the *duodenum*. In the duodenum, the particles are mixed with bile which is produced by the liver and stored in the gall bladder between the meals, and the alkaline pancreatic digestive juices containing lipase and proteases.

After the stomach, for efficient digestion by other enzymes, the pH must be raised and transit patterns be appropriate to the complete digestion of the meal during a single transit period. Some of the end products of digestion are relatively polar: amino-acids, mono-saccharides etc. and must be absorbed by active transport. They are valuable, so this will be achieved against an unfavourable

concentration gradient by expending energy. The length of the small bowel, consisting of the proximal jejunum and distal ileum, varies between 3 and 8 m (Teitelbaum et al., 2013), and provides a large surface area for absorption of nutrients, liquid and medicinal products.

Undigested products reach the colon through the ileocaecal valve. In the colon, bacterial fermentation of the undigested fibres will take place. After absorption of the excess liquid, the stools will be retained in the left hemicolon and the rectum until defecation upon relaxation of the internal and external anal sphincters.

This is the scenario for oral medication: effectively deliberately consuming a poison which for the most part must be absorbed into the systemic circulation. If the material is not recognised, then it must be small enough to be absorbed passively through transcellular and/or paracellular routes. If it is designed to mimic an essential component in the diet or part of the drug motif looks like a nutrient, then it may be handled by active transport and show saturability as part of the absorption process. Because drugs are partially ionised as weak bases or acids and will partition, regional absorption will occur where contact is sufficient for the drug in solution to be absorbed into membrane and where the pH yields sufficient material in a non-ionised form to be taken up into the enterocyte and then onwards to the systemic circulation and other organs.

The European Network on Understanding Gastrointestinal Absorption-related Processes (UNGAP) aims to improve our understanding of intestinal drug absorption by creating a multidisciplinary network of researchers from academia and industry engaging in scientific discussions. This review will summarize the current knowledge on anatomy and physiology of the human gastrointestinal tract with emphasis on human studies for the evaluation of the regional drug absorption and the prediction of oral dosage form performance.

2. The gastrointestinal mucosa

2.1. Regional mucosal differences

The gastrointestinal mucosa is the innermost structure of the digestive tract that influences digestion, absorption and secretion. Being in direct contact with the luminal content, the epithelial monolayer of the mucosa is important in regulating the passage of nutrients, ions, and solutes through paracellular and transcellular pathways. The epithelial cells are supported by a basement membrane composed of extracellular matrix components including laminins, collagens and proteoglycans, and act together as a barrier between the luminal content and the immune system. The underlying loose connective tissue is the *lamina propria*, which provides support and nutrition to the epithelium (Van Eyken et al., 2014). The functional differentiation of the GI mucosa differs both between and within the tissue of the stomach, small intestine and large intestine, leading to region dependent properties affecting drug absorption (Fig. 2).

The stomach has two functional regions: the oxyntic gland area (fundus and corpus) and the pyloric gland area (antrum). The oxyntic or acid secreting area of the stomach aids in chemical digestion, and is characterized by vertical tubular indentations consisting of an apical pit region, an isthmus and a gland (Schubert and Peura, 2008). The progenitor cells are located in the isthmus and can differentiate into all the gastric cell types while migrating, and establish an epithelial layer that is regenerated every 2–4 days (Creamer et al., 1961). Stem cells can differentiate into surface mucous cells while migrating upwards, and HCl-secreting parietal cells, pepsinogen secreting chief cells and enteroendocrine cells which migrate downwards (Schubert and Peura, 2008). The surface mucous cells secrete an adherent mucus layer that has a mean thickness of 180 μm , range: 50–450 μm (Jordan et al., 1998) (Newton et al., 2000) (Newton et al., 1998) (Kerss et al., 1982) (Al-Marhoon et al., 2005), which hydrates to form two distinct layers on the intestinal epithelium, protecting the stomach from self-digestion (Van Den Abeele et al. 2017a). Mucus is a hydrocolloid composed of water

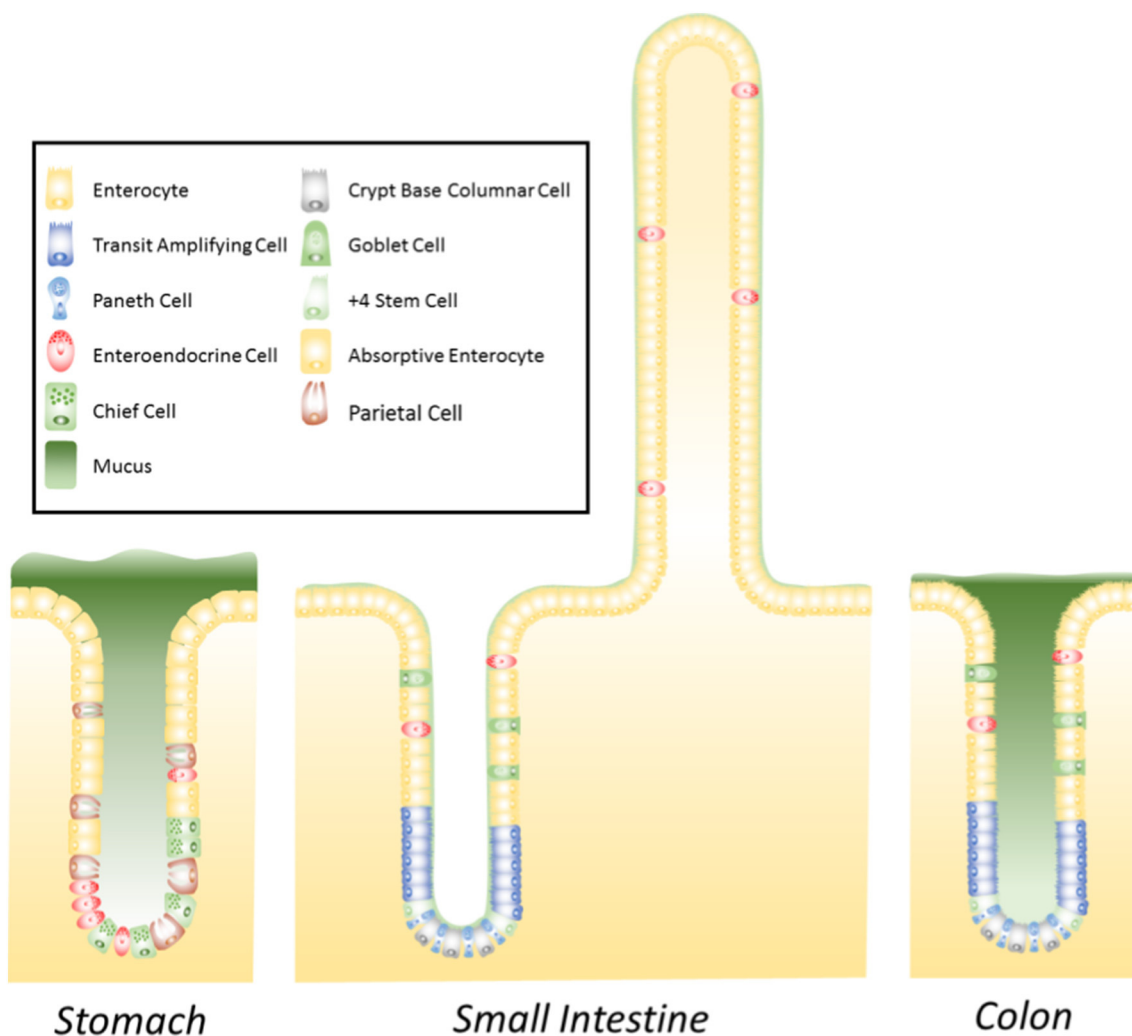


Fig. 2. The functional differentiation of the gastrointestinal mucosa.

(95%), lipids, electrolytes, and glycoproteins. The secretion of gel-forming mucins occurs in a region dependent manner along the GI tract (Kim and Ho, 2010; Teubl et al., 2013), with up to 20 different mucins. Gastric mucus is predominantly enriched with the gel-forming protein MUC5AC (Johansson et al., 2011), nevertheless mucins present in adjacent crypts can be differently glycosylated (Andrianifahanana et al., 2006; Kim and Ho, 2010). Interspersed between parietal cells are neuroendocrine, enterochromaffin-like cells (EC cells) which aid in the secretion of acid by release of histamine (Soybel, 2005). These represent 30–50% of enteroendocrine cells in the oxyntic mucosa (Zhao and Chen, 2012; Chu and Schubert, 2013). Enteroendocrine cells are the largest endocrine organ (< 1% of gut epithelial cells) of the human body (Chen et al., 1999; Schubert and Peura, 2008), and regulate secretion, motility, food intake and metabolism. A (and GR cells), G and D cells can be found in the stomach as well and secrete ghrelin, gastrin and somatostatin respectively. G and D cells are also present in the small intestine (Latorre et al., 2016).

Intestinal cells originate from multipotent LGR5⁺ stem cells in the base of glandular crypts (Loeffler et al., 1993; Barker et al., 2007) and have a high cellular turnover rate (Creamer et al., 1961). Stem cells of the small intestine give rise to a monolayer of enterocytes that is characterized by protrusions that extend into the gut lumen, called villi, resulting in a potential absorptive surface area of 60 m² in both the jejunum and ileum. In this way, the small intestine maximizes absorption of nutrients, in addition to aiding in chemical digestion. Villi are surrounded by epithelial invaginations termed the crypts of

Lieberkühn (where stem cells reside in the base) (Clevers, 2013), in contrast to the colon where the surface area totals around 0.25 m² as there are no villi (Kararli and Searle, 1995). The amount of goblet cells increases along the gastrointestinal tract, ranging from 4% in the duodenum to 16% in the distal colon (Cheng, 1974; Kim and Ho, 2010). The MUC2- rich mucus is a thin and discontinuous layer (Szentkuti and Lorenz, 1995; Bajka et al., 2015) in the small intestine that facilitates the absorption of a larger range of molecular nutrients, opposed to the thick two layer system in the colon (range: 20–52.5 μm) (Strugala et al., 2008). The colonic mucus consists of a stratified and sterile inner mucus layer, which can be reformed by endogenous proteases into a thicker and more porous outer (loose) mucus layer to allow a symbiotic relationship with microbiota (Johansson et al., 2011; Sellers and Morton, 2014). The loose mucus can be degraded by bacteria, thereby facilitating the passage of ingesta (Birchenough et al., 2015). An intact colonic mucus appears to be completely clear from bacteria in a zone of about 30 μm which does not stain. The underlying mucosa remains immunologically quiescent (Swidsinski et al., 2007). Johansson and colleagues have also identified the two-layer structure of colonic mucus with an inner zone devoid of bacteria and an upper zone that is colonised by bacteria and varies in thickness as mucin is digested (Johansson et al., 2008). The mucus of the small intestine and caecum however is enriched with antimicrobial peptides from Paneth cells (Ayabe et al., 2000; Van Eyken et al., 2014), thereby protecting it not only from physical and chemical stress, but also exerting a bactericidal effect. EC cells in the small intestine can either be ‘open cells’, where

the microvilli are in direct contact with the gut lumen, or ‘closed cells’ that are indirectly regulated through neural and humoral mechanisms (Sternini et al., 2008). Apart from D and G cells in the small intestine, this area is also typified by I and K cells that secrete cholecystokinin and gastric inhibitory polypeptide respectively, while both the small intestine and colon produce 5-HT. L cells are located in the distal small intestine and colon, with GLP-1, GLP-2, PYY and 5-HT identified as the main products (Latorre et al., 2016).

2.2. Region dependent drug absorption

Oral drug absorption is region dependent and is influenced by the mucus layer, intercellular tight junctions, and the expression of transporters and enzymes in epithelial cells.

It has been postulated that electrostatic interactions and the pore size of the mucin network can affect the mobility of particles, especially molecules with a higher molecular weight including peptides and DNA (Kirch et al., 2012; Bajka et al., 2015). Firstly, mucus can limit the absorption rate and bioavailability due to the chemical interactions between glycoproteins and the drug. Hydrophilic drugs generally permeate freely through the mucus layer, but permeation can be hindered at a pH > 2.6 due to electrostatic interactions between the negatively charged mucins and the positively charged drugs. Drugs can also show affinity for the hydrophobic protein core of mucins or for hydrogen bonding (Varum et al., 2012). Secondly, gel-forming mucins can have size filtering properties. The mucus mesh size of human salivary mucin fibres was determined as around 0.8 μm (Teubl et al., 2013) which was similar to the pore size of porcine mucin in the stomach. It was noted that nanoparticles cannot always reach the membrane of the epithelial cells, as they get captured in the glycocalyx. However, by developing mucoadhesive systems that surpass the mucus turnover, the epithelium can still absorb the drugs released from the nanoparticles (Huckaby and Lai, 2018). In addition, PEGylated nanoparticles have been shown to diffuse rapidly in physiological mucus, approaching similar fluxes through water (Lai et al., 2007). The thickness and composition of mucus differs regionally, which suggests a slowed-down diffusion and a lower permeability through the mucus, but also implies that it is region dependent (Falavigna et al., 2018).

The passive paracellular absorption of drugs is dependent on the expression and regulation of tight junctions, which are found in the most apical part of the junction complexes that connect neighbouring epithelial cells. Tight junctions are expressed differentially between stomach, small bowel, and large bowel (Rahner et al., 2001; Hewitt et al., 2006; Lu et al., 2010; Lu et al., 2013). This results in a barrier that is relatively leaky in small intestinal tissue, while gastric and colonic mucosa are of intermediate tightness. The differential expression of claudins between regions is the main determinant of these barrier properties, with claudin-1, -3, -4, -7, and -8 being expressed most prevalently in the colon, and claudin-2, -7, -8, and -9 in the upper intestinal tract. Claudins can be selective for cations (claudin-2, -10b, -15), or anions (claudin-10a, -17), water (claudin-2) or not selective at all (claudin-4, -8, -14) (Krug et al., 2014). Sjöberg and colleagues showed that highly permeable lipophilic drugs tend to have the same or higher permeability coefficients in the colon in comparison with the small intestine, while the reverse is true for more polar compounds (Sjöberg et al., 2013; Peters et al., 2016).

Efflux pumps at the (apical) membrane can play pivotal roles in the transcellular absorption, distribution and excretion of drugs by facilitating the excretion of xenobiotics and endogenous compounds. In the intestine, ATP-binding cassette (ABC) transporters are among the most highly expressed transporters (colon: P-gP (5%), BCRP (3%), MRP2 (25%), small intestine: P-gP (8%) BCRP (4%), MRP2 (10%)) (Drozdziak et al., 2014) and together they function as a significant barrier to intestinal absorption, especially as they have a partially overlapping substrate spectrum (Misaka et al., 2013). The abundance of P-gP increases from the proximal to the distal region of the small intestine,

while the opposite is true for CYP3A4 (Drozdziak et al., 2014; Li et al., 2016). Due to the synergistic interplay of CYP3A and P-gP on dual substrates and the long intestinal transit time, compounds can undergo extensive presystemic intestinal metabolism (Siissalo and Heikkinen, 2012; Peters et al., 2016). However, so far site-dependency has only been confirmed for substrates of P-gP. Furthermore, region dependent uptake can be attributed to differences in intracellular metabolism, with higher rates of intracellular metabolism leading to sink conditions inside the cells (Peters et al., 2016).

3. The unstirred water layer

The unstirred water layer (UWL) exists above the epithelium as a stagnant boundary layer of varying dimensions. It is a general phenomenon of wetted objects, even on non-biological surfaces (Green and Otori, 1970) and for the gut forms part of the barrier for molecules diffusing across from the luminal space into the cell. The relative contribution of the unstirred water layer in measurements of drug flux is known to vary in different *in vitro* preparations and in clinical trials under different conditions, especially as a function of mixing and because motility changes occur along the gut, it contributes to measurements of regional drug absorption in the gut.

In early physiological experiments, amphibian preparations were widely used by physiologists as frogs were readily available and cold-blooded so easy to use in a laboratory environment. The dimensions of the UWL on frog skin were determined by Dainty and House (1966), using potassium sulphate or sodium sulphate as a probe. The measurement indicated a layer within the dimensions of 120–230 μm for potassium ions dependent on stirring conditions but the analysis ignored the contribution of fixed surface charges. Sodium sulphate gave a value of around 30–50 μm , suggesting that the resistance to transport of the sodium ion was much nearer to the surface. Similar values and differences were calculated under moderate stirring conditions by others for the same tissue (Kidder et al., 1964). Dainty and House also noted that the resistance to sodium movement occurs within the membrane, as had been noted by Winn's group (Dainty and House, 1966; Winn et al., 1964). Winn reported that when an epidermis becomes vacuolated under osmotic drive, the sodium diffusion coefficient increased ten-fold. This suggested to Dainty and House, that a predominant barrier to solute diffusion might also lie in the cytoplasm (Dainty and House, 1966). Thus, there would be inner and outer UWL resistance as discussed later by Westergaard and Dietschy (1974).

In well culture systems, the apparent UWL measurements above the planar surface of the well can extend to 1200–1500 μm . Transport rates are routinely normalised to the surface area of the confluent monolayer and since even vigorous shaking in a well culture system leaves a UWL thickness of between 500 and 1000 μm , the true Michaelis constant of an enzymatic process, K_t , may be elusive. For example, Shibayama and colleagues report that the common methods of estimating the kinetics of the organic cation transporters OCT and MATE1 by Chinese hamster ovary cells yields overestimates the true K_t by two to ten-fold (Shibayama et al., 2015). Hidalgo and colleagues have described increasing mixing by gas lift to calculate the mixing forces needed to decrease the UWL in trans-well cell culture. As the UWL was reduced from 1966 to 564 μm , an increase in the diffusion rate constant of testosterone from $3.08 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ under stagnant conditions to $14.08 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ at a flow rate of $40 \text{ mL} \cdot \text{min}^{-1}$ (Hidalgo et al., 1991) was noted.

If a UWL overlays a tissue with geographic tortuosity, that it is to say it is highly folded at microscopic dimensions or is within an unstirred matrix as would be found in spaces between and under cells, the effects of water stagnancy become more evident. Conversely, if the surface layer is subjected to shear on a relatively flat surface, such as a flat mounted rat jejunum, the UWL thins dramatically (Thomson and Dietschy, 1980).

In the gut, Winne showed that the uptake rates of butanol,

antipyrine, salicylic acid and urea were increased in a perfused rat jejunal loop. This was attributed to alteration of the UWL and to increased surface area for absorption (Winne, 1978). Winne calculated that the UWL contributed between 10 and 36% of transport resistance. Small molecules shuttle in the intracellular space (active transport between cells) or are transiently displaced during an action potential. These processes may be coupled to water flow - for example overall flux is assisted by the sodium-glucose linked cotransporter, SGLT1 which is a highly efficient engine for passive water movement across the intestine (Erokhova et al., 2016).

3.1. Measurements of UWL

The UWL in the gut has been described as two regions: that on the apical surface and that between villi (Westergaard and Dietsch, 1974). With stirring, the apical layer in a flat surface mounted rabbit jejunum shear thins. The change in UWL is calculated by measuring the $t_{1/2}$ in transmucosal potential difference change following the exposure to a hyperosmotic solution. The time to achieve the new steady state is a function of the UWL thickness and diffusivity of the probe molecule (Diamond, 1966). Barlow and colleagues have recently described a rheological device based on measurement of a droplet interface bilayer (Barlow et al., 2017). Di Cagno and colleagues have measured the diffusivity by time-resolved UV measurements quantifying the movement of a concentrated solution at the base of the well of a cuvette and monitoring changes in absorbance over a 24-hour period (di Cagno et al., 2018).

Calculation of the UWL of gut segments *in vivo* often assumes a measurement based on a smooth cylinder surface area. The gut is highly folded and has villus projections contributing to kVF, the surface expansion factor. This is a measure that calculates the contribution of the folds of Kerckring to the expansion of the luminal surface area, compared to a simple tube. In a recent paper by Lozoya-Agullo and colleagues, the figure cited is a kVF of 4.6. This would indicate that the UWL calculated by one of her co-authors Dr. Marival Berjemo of 103 μm (Bermejo et al., 2004) for a closed perfused rat loop (known as a Dolutio preparation), would be 2506 μm (Lozoya-Agullo et al., 2018). In man, the more folded intestine results in a kVF of value of 33 versus a kVF of 2.9 to 3.1 in rats, due to higher density of villi and the presence of plaecal folds in the human intestine which are not present in rat (DeSesso and Jacobson, 2001). Read and colleagues attempted to measure the thickness of the unstirred water layer and electrogenic glucose absorption using a multi-lumen tube by sequentially infusing solutions containing sodium chloride, mannitol and glucose (Read et al., 1977). Measurements were made on healthy controls and patients with coeliac disease who had active status or who had been treated. The data indicated a mean thickness in healthy adults of $632 \pm 24 \mu\text{m}$, decreasing to $442 \pm 23 \mu\text{m}$ in patients with active disease. The thickness of the UWL in treated coeliac patients was nearer to healthy controls ($585 \pm 49 \mu\text{m}$). Levitt and colleagues calculated the unstirred water layer from measurements of the hydrolysis of the disaccharides, sucrose and maltose. The measurements suggested that the unstirred diffusion layer of the human jejunum was only around 35 μm . Although a UWL of 600 μm as estimated from electrogenic measurements would be rate limiting, epithelial cell function and luminal stirring would be more likely barriers if the UWL were so thin (Levitt et al., 1992). Static systems are to a large extent unrepresentative of events likely to be encountered *in vivo*. In any case, in seemingly poorly stirred systems, stochastic events occur. Barry & Diamond observed that even on flat surfaces, water layers will be subject to convective flow due to solute gradients, small temperature gradients and water density gradients (Barry and Diamond, 1984). The authors suggest that when active and passive transport of a solute is balanced, the concentration in the UWL will be the same as in the bulk phase; however, if the solute is transported out of the cell the UWL concentration increases. The consequence for efflux transport from the Barry & Diamond perspective is

that the concentration-driven gradient for the solute would be decreased. Changing the architecture of the mucosal membrane by stretching causes shortening and swaying movements of the villi (Lee, 1983) although larger movements of the intestine are more effective in changing the absorptive surface. Smaller canine jejunal biopsy samples, which were stretched showed increased water absorption suggesting changes in the sub-villous architecture facilitates water removal by the lymphatic system. Increasing villus motility by mechanical agitation was also investigated in sections from along the canine gut by Mailman and colleagues, who reported that this was without effect on the UWL (Mailman et al., 1990). An increase in perfusion rates in the partially obstructed rat ileal preparation from 1 to 100 mL/min increases the absorption of glucose by 150% which was attributed to increased mucosal surface area (Lewis, 1975). Conversely, a distension of the intestine was found to increase the inter-villus width, without an effect on total surface area (Scott-Harris et al., 1988).

3.2. Agitation, UWL and the movement of molecules

The absorption of BCS class II drugs is limited by transport of the dissolved species through the UWL since permeation through the membrane is rapid for highly lipophilic drug such as danazol (Kataoka et al., 2012). Incorporating the drug in a system that facilitates molecular dispersion or promotes low interactivity e.g. shields long range interactions caused by ionization, reduces the interactions within the UWL and increases the rate of permeation. The link between permeation and solubility driving the thermodynamic activity of a drug, classically determines the proportional rate of transport of a drug (Higuchi, 1977). Later researchers have commented that the flux can be alternatively considered as the interplay between solubility and permeability ignoring thermodynamic arguments (Dahan et al., 2016; Beig et al., 2017a). As an example, generating a large solubilised species such as a micelle will impact inherent diffusivity rates and reduce the free concentration in the unstirred water layer. In some situations, the influence of the membrane efflux pump will dominate and be highly non-linear and any influence of the UWL will not be apparent. This is true for the BCS class IV molecule rifamixin, a rifampicin analogue. Dahan and colleagues described the influence of a supersaturated rifamixin formulation on rat intestinal absorption of the drug. At $1 \times$ and $100 \times$ supersaturation, fluxes were similar whereas at $250 \times$ supersaturation absorption rose; however, this effect was not apparent in an artificial PAMPA membrane (Dahan et al., 2016), since both solubility and permeability were increased by the amorphous solid dispersion (Beig et al., 2017b).

Within the dissolving formulation, a temporary association of drug with excipients and natural gut secretions will form complex structures that would be slow to diffuse across the UWL and therefore it is unsurprising that agitation in Caco-2 systems increases the movement of very large constructs. Kono and colleagues demonstrated that the UWL in culture was a significant barrier to the efficiency of transfection with a liposomal /pDNA complex (Kono et al., 2016).

3.3. The role of mucus in the UWL

The surface mucins extend 200 to 1500 nm above the epithelium anchored to the glycocalyx, an integral part of the plasma membrane. The external surface expresses glycoproteins and glycolipids, attached to adherent mucins to form the firmly adherent mucin layer (30 μm). The mucins hydrate, swell and become more loosely adherent becoming sheared by peristalsis (Atuma et al., 2001; Kim and Ho, 2010). Where shear forces are lower and there is a higher density of goblet cells, i.e. in the colon, the thickness increases markedly up to 1.5 mm. Thus, a principle effect of mucin secretion is to increase the thickness of the UWL.

The thickness of the UWL within the mucus will vary as muscular movements through the colon are sluggish. Although scientists are very

familiar with the protective role of the mucus in the colon, they may have ignored the small intestine. Here intestinal mucus may also have an important role to protect the villus against insult (Bryan et al., 1980) and secretion is increased by goblet cell capping: the process by which the apical concentration of goblet cells increases. There is no doubt that an attached mucoid layer will support the UWL in the same manner as other large hydrophilic polymers as described in the experiments of Jenkins and colleagues and those of Johnson and Gee (Jenkins et al., 1978; Johnson and Gee, 1981, 1982).

The effects of artificially supplementing the barrier properties of the UWL have been considered by nutritionists, particularly in the treatment of obesity. Possible clinical advantages of dietary fibre in the management of oral glucose loads for diabetic patients were first reported by Jenkins and colleagues. They compared various fibres and drew attention to the role of guar gum and pectin in mediated postprandial glycaemia (Jenkins et al., 1978). The effect of viscous polymers on absorption of sugars was further explored by scientists at the Agriculture Research Centre in Norwich, a group that did much research on the benefits of fibres in diet though the 80's. Using everted gut sacs, it was shown that enhanced viscosity reduced stirring in the intervillous compartment slowing presentation to the membrane surface enzymes. It was also hypothesized that the increased UWL thickness produced a partial anoxia (oxygen starvation) in the preparation, reducing the generation of ATP to drive active transport (Johnson and Gee, 1982). Blackburn and Johnson examined the effects of guar gum, diluted in saline and found that the system markedly increased the UWL, resulting in a resistance to absorption (Blackburn and Johnson, 1983). Smithson and colleagues attempted to separate the barrier properties of the UWL from the mucus layer component and concluded that the UWL had a lesser role than the secreted mucus as large molecules, including the probe [125 I]-labelled lactoperoxidase, could not get access to the membrane of the intact enterocyte layer due to the presence of the mucus coat (Smithson et al., 1981).

Secretions produced near a wall will aid mixing since the chyme is a weak gel which forms a matrix when static. When secretions are watery, shear induced by muscular motion of the intestinal wall produces flow; conversely, if fluid content is a stronger gel or contains more particulates, the mass in the lumen will show elastic distortion and reestablishment (Lentle and De Loubens, 2015). The authors also comment that the individual movement of villi do not contribute to mixing but rather it is the villus crowding that occurs as peristaltic movements of circular and longitudinal muscle causing a distortion of the lumen.

3.4. The UWL acid microclimate

It has long been established that the surface layer on top of the mucosa is acidic even when bulk buffer pH is near to neutrality as shown in everted gut sacs with a 60 μ m electrode (Lucas et al., 1975). Lucas also reported the acid microclimate in vivo, using anaesthetized rats with an exteriorized jejunal loop bathed with Krebs-Heinsleit bicarbonate buffer at pH of around 7.2. The pH in the UWL as the probe microelectrode was advanced to the surface using a micromanipulator and was shown to approach a pH around 5.9 (Lucas, 1983). The surface pH became alkaline in the ileal segment, becoming slightly more alkaline than the perfusing buffer. During anoxia, in the presence of glucose, the jejunal tissue microclimate remained acidic whereas in the absence of glucose, the acid microclimate drifted back to normal. It appears that during periods of anoxia, the gut uses apical transporter pumps protonated metabolites (such as lactate) into intestinal lumen. Note that Caco2 monolayers are unable to support this acid microclimate which has to be mimicked in culture to obtain more representative behaviour (Gleeson et al., 2017).

4. The intraluminal environment

4.1. Physicochemical characteristics and intraluminal composition

In oral drug absorption studies, the fasted state is typically defined as the period immediately after the ingestion of a glass of water and until the consumption of the next meal, provided that the water is consumed approximately 12 h, after the last meal (Food and Drug Administration, 2002; European Medicines Agency, 2010). The fed state, on the other hand, is typically defined as the period from 30 min after the start of consumption of a high-fat, high-calorie (800–1000 kcal) meal until the stomach is practically emptied from meal components, provided the meal is consumed 12 h, after the last one (Food and Drug Administration, 2002; European Medicines Agency, 2010).

4.1.1. Stomach

The stomach is the first organ in which intense and prolonged contact between an orally ingested formulation and any gastrointestinal fluids takes place. Inevitably, this environment will affect local formulation and drug behavior. Gastric fluids consist of a variety of components that are either secreted by the stomach (e.g. gastric acid, enzymes, electrolytes, mucus), swallowed/ingested (e.g. saliva, food, liquids) or refluxed from the duodenum in the stomach (e.g. bile constituents). In this regard, the stomach is a somewhat special case as the physicochemical characteristics of its contents are directly affected by anything ingested. Hereafter, the physicochemical characteristics of the gastrointestinal environment are discussed in the fasted state, after ingestion of a glass of water and in the fed state. In general, ingestion of water can be viewed as a temporary dilution of gastric contents. Ingestion of a meal will initially result in a gastric fluid composition that closely resembles that of the meal. During digestion and gastric emptying of that meal the gastric environment will gradually change back to baseline (fasted) conditions. The composition of these stomach fluids will affect formulation disintegration and drug dissolution and/or solubility. Moreover, the high variance of these contents will install variability local and systemic drug disposition.

In general, the gastric environment is reported as typically acidic due to the secretion of hydrochloric acid by parietal cells lining the gastric wall. Median gastric pH values between 1.5 and 1.9 have been measured (Pedersen et al., 2000; Pedersen et al., 2013; Lindahl et al., 1997; Press et al., 1998; Dressman et al., 1990). However, a wide range of pH values was observed (1.4 to 7.5). In the fundus, the lower density of parietal cells gives rise to a pH shift to between 2.2. and 4. Low pH values will be recorded if the probe penetrates mucus as the contribution of surface bicarbonate is reduced. High pH values measured in gastric samples likely result from swallowing saliva and/or sporadic duodenogastric reflux. After water ingestion, pH values may vary between 1.6 and 3.3 although initial gastric pH exhibited large inter-individual differences with values ranging from pH 1–8 (Kalantzi et al., 2006; Koziolok et al., 2014a; Petrakis et al., 2015). In the postprandial state, the ingested meal volume and composition will be the main determinants of initial postprandial pH (Koziolok et al., 2013; Kalantzi et al., 2006; Dressman et al., 1990) The timeframe for re-acidification will depend on the buffer capacity of the administered meal as well as the rate at which the meal is emptied from the stomach (Kalantzi et al., 2006; Dressman et al., 1990; Simonian et al., 2005). Dressman and colleagues for example reported a timeframe of < 2 h to reach pH 2 in the postprandial stomach after ingestion of a solid meal (1000 kcal) (Dressman et al., 1990).

The average buffer capacity of gastric contents in the fasted state have been reported as $14.3 \pm 9.5 \text{ mmol}\cdot\text{L}^{-1}\cdot\Delta\text{pH}^{-1}$ ($N = 19$) (Pedersen et al., 2013). Water intake will dilute gastric content, resulting in a temporary decrease in buffer capacity (Kalantzi et al., 2006; Litou et al., 2016). Following a meal, the buffer capacity of gastric content is expected to initially be similar to that of the ingested meal.

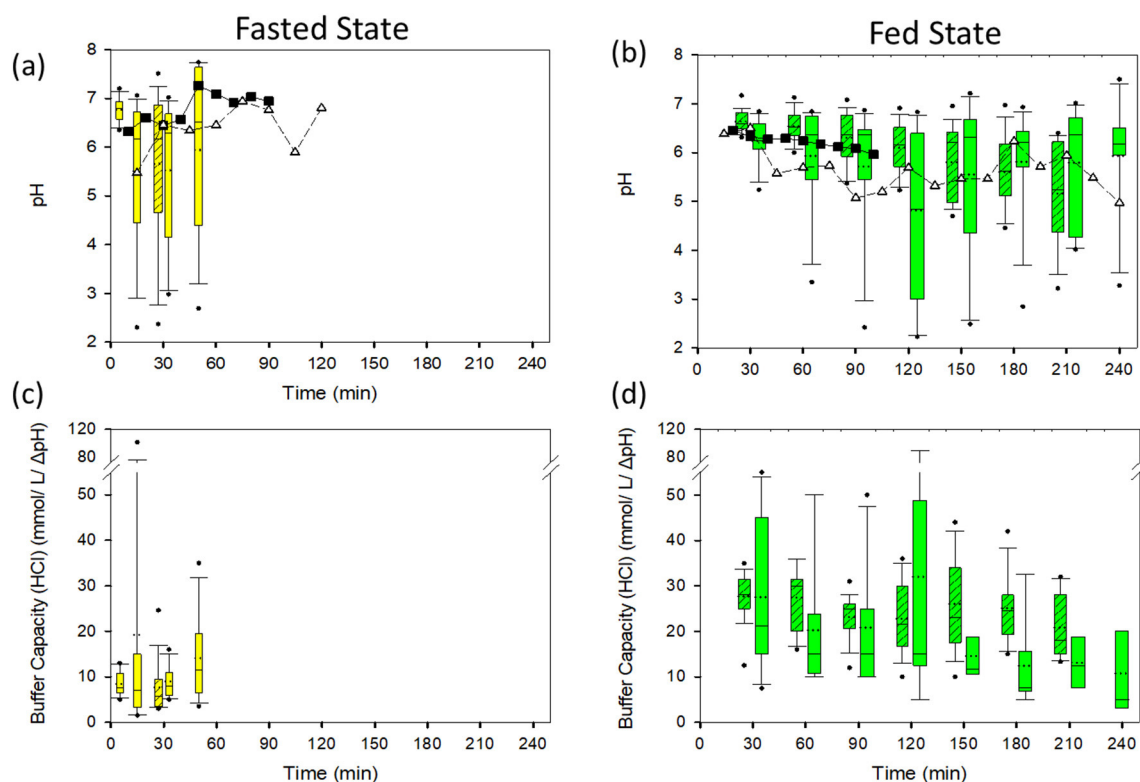


Fig. 3. pH (a and b) and buffer capacity (c and d) data collected immediately upon aspiration of samples from the upper intestine at various times after administration of a glass of water to fasted adults (fasted state, a and c) and a homogenous or heterogenous liquid meal to fasted adults (fed state, b and d). Only studies which did not involve co-administration of an ionizable drug were considered.

Fasted state: empty boxplot (Litou et al., 2016); lined boxplot (Kalantzi et al., 2006); solid squares (Riethorst et al., 2016; mean values); open triangles (Clarysse et al., 2009; median values).

Fed state: empty boxplot (Vertzoni et al., 2012 - heterogeneous liquid meal); lined boxplots (Kalantzi et al., 2006 - homogeneous liquid meal); solid squares (Riethorst et al., 2016 - homogeneous liquid meal; mean values); Open triangles (Clarysse et al., 2009 - homogeneous liquid meal; median values).

Kalantzi et al. for example observed buffer capacity's ranging from 14 to 28 $\text{mmol}\cdot\text{L}^{-1}\cdot\Delta\text{pH}^{-1}$ following the ingestion of a liquid meal. These values are close to the buffer capacity of the meal itself (i.e., $\text{mmol}\cdot\text{L}^{-1}\cdot\Delta\text{pH}^{-1}$) (Kalantzi et al., 2006).

Viscosity measurements in human gastric aspirates are characterized by large variability within and between studies. The viscosity of fasted gastric aspirates has been reported in ranges from 1.7 to 9.3 mPa·s (shear rate = 50 s^{-1} , $N = 17$). Gastric aspirates are observed to be inhomogeneous resulting in marked intra-sample variability (Pedersen et al., 2013). Upon meal intake, gastric viscosity initially increases, depending on meal composition, and subsequently declines due to dilution of gastric content resulting from stimulated gastric secretion (Abrahamsson et al., 2005; Marciari et al., 2000; Radwan et al., 2014).

Gastric fluids contain important amounts of enzymes, which are mainly involved in the digestion of meal components. Two of the main digestive enzymes present in the stomach are pepsin and gastric lipase. Pepsin is secreted as the inactive pepsinogen, which is converted to pepsin in the presence of luminal gastric acid ($\text{pH} < 5$) (Soybel, 2005; Gritti et al., 2000; O'Connor and O'Moráin, 2014). The activity of pepsin has been reported to be maximal at pH 2 and non-existent at pH 5.5 or higher (Johnston et al., 2007). Furthermore, pepsinogen output has been observed to significantly increase after ingestion of a meal (Kalantzi et al., 2006). In a similar way, gastric lipase is only active within a specific pH range with a maximal activity reported between pH 3–5.5. As enzymatic activity is most likely either very low or non-existent in fasted state, mainly data on gastric lipase activity in fed human subjects have previously been reported in literature (Pedersen et al., 2013; Ville et al., 2002; Williams et al., 2012). Interestingly, reports indicated a considerably more extensive digestion of

triglycerides in the stomach after intake of a liquid meal compared to solid meals, due to a better pre-emulsification of dietary lipids in a liquid meal (Carrière et al., 2001; Renou et al., 2001; Carrière et al., 2005; Borgstrom et al., 1957; Carrière et al., 2000; Bergström et al., 2014).

Surface tension is mainly influenced by the presence of pepsin and the sporadic reflux of duodenal content in the stomach (Pedersen et al., 2013; Efentakis and Dressman, 1998; Bergström et al., 2014). In fasted volunteers, values of $34.8 \pm 5.2\text{ mN}\cdot\text{m}^{-1}$ ($N = 19$) and 35 to $45\text{ mN}\cdot\text{m}^{-1}$ ($N = 8$) are reported (Pedersen et al., 2013; Efentakis and Dressman, 1998). Slightly higher surface tension is observed in gastric fluid from volunteers ingesting water (Kalantzi et al., 2006). Furthermore, Kalantzi et al. observed surface tensions 30% lower after intake of a liquid meal (Kalantzi et al., 2006).

The presence of proteins (e.g., pepsin) and electrolytes determines the osmolality of gastric content. Without prior water intake, mean osmolality (\pm SD) of human gastric aspirates has been reported to be $191 \pm 36\text{ mOsm}\cdot\text{kg}^{-1}$ ($N = 36$) and $220 \pm 58\text{ mOsm}\cdot\text{kg}^{-1}$ ($N = 19$) (Lindahl et al., 1997; Pedersen et al., 2013). Several authors have observed a considerable decrease in osmolality of gastric aspirates immediately after water intake. For instance, 10 min after water administration, Petrakis et al. measured an osmotic value of $32\text{ mOsm}\cdot\text{kg}^{-1}$ in pooled gastric aspirates (Petrakis et al., 2015). After meal ingestion, the properties of the meal will dictate osmolality of gastric fluids in fed subjects. For instance, 30 min after intake of a liquid meal, osmolality of gastric content was found to be similar to that of the meal as such (559 vs. $610\text{ mOsm}\cdot\text{kg}^{-1}$, respectively) and to decrease to fasted state values within 3.5 h as the meal is emptied from the stomach (Kalantzi et al., 2006).

4.1.2. Upper intestine (duodenum and proximal jejunum)

Unlike in the fasted state, to date, the physicochemical characteristics and composition of contents of upper intestine in the fed state have been investigated only to a limited extent, partly due to difficulties in sampling from the region. Data have been collected, after administration of liquid meals having caloric content and origin of calories similar with those in meals which are typically administered in oral drug absorption studies (Pentafragka et al. 2019). Information from studies which did not involve co-administration of agents which could potentially affect the luminal environment (e.g. ionizable drugs) is summarized below.

The average pH is slightly acidic in the fasted state with median values from various studies ranging between 6.1 and 7.0. However, at least during the first hour after administration of a glass of water variability is high and pH values as low as 3 can occur (Fig. 3a). In the fed state, data collected after administration of homogenous or heterogeneous liquid meals indicate slightly decreased pH values during the course of digestion with the medians ranging between 4.8 and 6.5 during the first 3 h post meal administration (Fig. 3b). It is interesting to note that during the first hour post meal administration data variability is lower than during the first hour after administration of a glass of water (Fig. 3a vs. b).

Median values ranging from 5.7 to 11.5 mmol/L/ Δ pH have been reported for the buffer capacity of contents in upper intestine immediately upon aspiration of samples in the fasted state (Fig. 3c). Corresponding values in the fed state are on average more than double, and, apparently, only slightly affected by the course of digestion (Fig. 3d). It should be noted that buffer capacity measurements should be performed immediately upon aspiration of samples, because of the instability of the bicarbonate-CO₂ buffer (the main buffering species in the fasted state) and the potential continuation of digestion of meal components in the fed state during handling and storage of the aspirated samples.

Although variable in total, sodium and chloride concentrations are almost equivalent, about 100 mM on average, both in the fasted and in the fed state (e.g. Pentafragka et al. 2019). Potassium concentrations are about 10 times lower and calcium concentrations are minimal with the potential impact of food intake on luminal concentrations of these two cations, unclear. Duodenal contents are hypo-osmotic in the fasted state (median range: 115–206 mOsm/kg) but they are mostly hyperosmotic in the fed state; median values at various times during the first 3 h after meal administration range from 215 mOsm/kg (at 3 h) to 423 mOsm/kg (at 2 h). Duodenal surface tension seems to be slightly higher in the fasted state; reported median values range from 32.7 mN/m to 35.3 mN/m in the fasted state and from 30.2 mN/m (at 3 h) to 35.1 mN/m (at 0.5 h) in the fed state. Total bile salt content is variable but, on average, it is higher in the fed state; median values from various studies in the fasted state range from 3.7 mM to 7.7 mM whereas overall median values during the first 3 h, after the administration of a liquid meal, range from 3.7 mM (at 3 h) to 18.2 mM (at 1 h). A median value of 11.5 has been reported for the ratio [total bile salt content/phospholipids] in the fasted state and a value of about 3.4 for the fed state. Cholesterol levels and variability increase after meal intake; median values in the fasted state range from 0.08 mM to 0.44 mM whereas median values at various times up to 3 h after meal administration range from 0.30 mM (at 3 h) to 3.12 mM (at 1 h). Glyceride, free fatty acid and carbohydrate content should be considered with caution as relevant concentrations are minimal in the fasted state and exclusively dependent on the specific meal composition in the fed state. Information on total protein content has been collected using kits and should also be considered with caution. The protein content in the fasted upper intestine reflects primarily the presence of pancreatic enzymes. A 5- to 10-fold increase in pancreatic lipase and a 5-fold increase in phospholipase-A2 are observed in the fed state (Riethorst et al., 2016). Since food intake reduces the mean fluid volumes in the small intestine (Schiller et al., 2005) and pH values are only slightly affected,

substantially higher enzyme activity is expected in the upper intestine in the fed state.

4.1.3. Lower intestine (distal ileum and proximal colon)

The emphasis to date has been on the physicochemical characterization of the environment in healthy adult upper GI tract as the oral drug absorption is usually complete in the upper small intestine. However, differences in the luminal environment between the upper small intestine and the lower intestine (distal ileum and proximal colon) may impact the performance of orally administered products which deliver drug during residence in the lower intestine. More specifically, in cases where an extended release or a colon targeting product is administered or where the drug has low permeability in the upper intestine but can be absorbed to some extent during its residence in the lower intestine, the environment in the lower intestinal lumen will influence drug/drug product performance (Diakidou et al., 2009).

Over the last decade, a well-defined protocol for direct sampling from the lower intestine with minimal effects on its physiology has been proposed and this provided a basis for the physicochemical characterization of contents collected from the lower intestine under conditions to which drugs/drug products are exposed during BA/BE studies in healthy adults (Diakidou et al., 2009; Reppas et al., 2015). Samples have been collected, five hours after the administration of a glass of water or after the consumption of the high-fat, high-calorie meal [as proposed by regulatory agencies (Food and Drug Administration, 2002; European Medicines Agency, 2010)] i.e. when drugs administered as conventional products or multiparticulate modified release products are expected to reach the lower intestine after oral administration.

In the distal ileum, the pH is slightly alkaline (median pH 8.1) regardless the dosing conditions. In the proximal colon (cecum and ascending colon) the pH in the fasted state is about 7.8 whereas in the fed state it is lower, about 6.0 (Fig. 4A), due to the increased bacterial fermentation activity after the consumption of the meal [Koziolek et al., 2015]. As with pH, buffer capacity does not differ between prandial states in distal ileum but it differed in the proximal colon. Compared to caecal and ascending colon contents, ileal contents have significantly lower buffer capacity only in the fed state (Fig. 4B). It is interesting to note that buffer capacity of ileal contents is similar with values reported for duodenal and jejunal contents in the fasted state (Diakidou et al., 2009; Reppas et al., 2015). In line with the lower pH values and the higher buffer capacity of caecal contents compared to ileal contents, total short chain fatty acid (SCFA) concentration is higher in cecum than in distal ileum in both prandial states. Although the presence of SCFAs did not differ between prandial states in these two regions, a significant food effect was observed in the contents of ascending colon (Fig. 4C).

Osmolality of ileal contents in the fasted state is lower than in the fed state [mean (SD) 60(50) vs 252(245) mOsmol/Kg] but osmolality of caecal and ascending colon contents do not differ between prandial states (Fig. 4D) (Diakidou et al., 2009; Reppas et al., 2015). Lower intestinal contents are generally hypoosmotic with values lower than in the duodenum in both prandial states (Pentafragka et al., 2019).

Protein content is lower in the fed state samples but total carbohydrate concentrations are higher (Fig. 4E and F). This can be explained by the fact that digestion and absorption of peptides administered with the meal is almost complete by the end of the first meter of the small intestine but carbohydrates administered with the meal may or may not be digested in the small intestine and may therefore reach the colon (Pentafragka et al., 2019). It has been reported that total carbohydrate content in the fed colon was approximately 30% of that observed in the fed upper small intestine (Kalantzi et al., 2006; Diakidou et al., 2009; Reppas et al., 2015).

In the ileal and caecal contents, differences in bile salt concentrations between the prandial states are not marked but in the contents of the ascending colon bile salt concentrations are higher in the fed than in the fasted state (Diakidou et al., 2009; Reppas et al., 2015). In all cases,

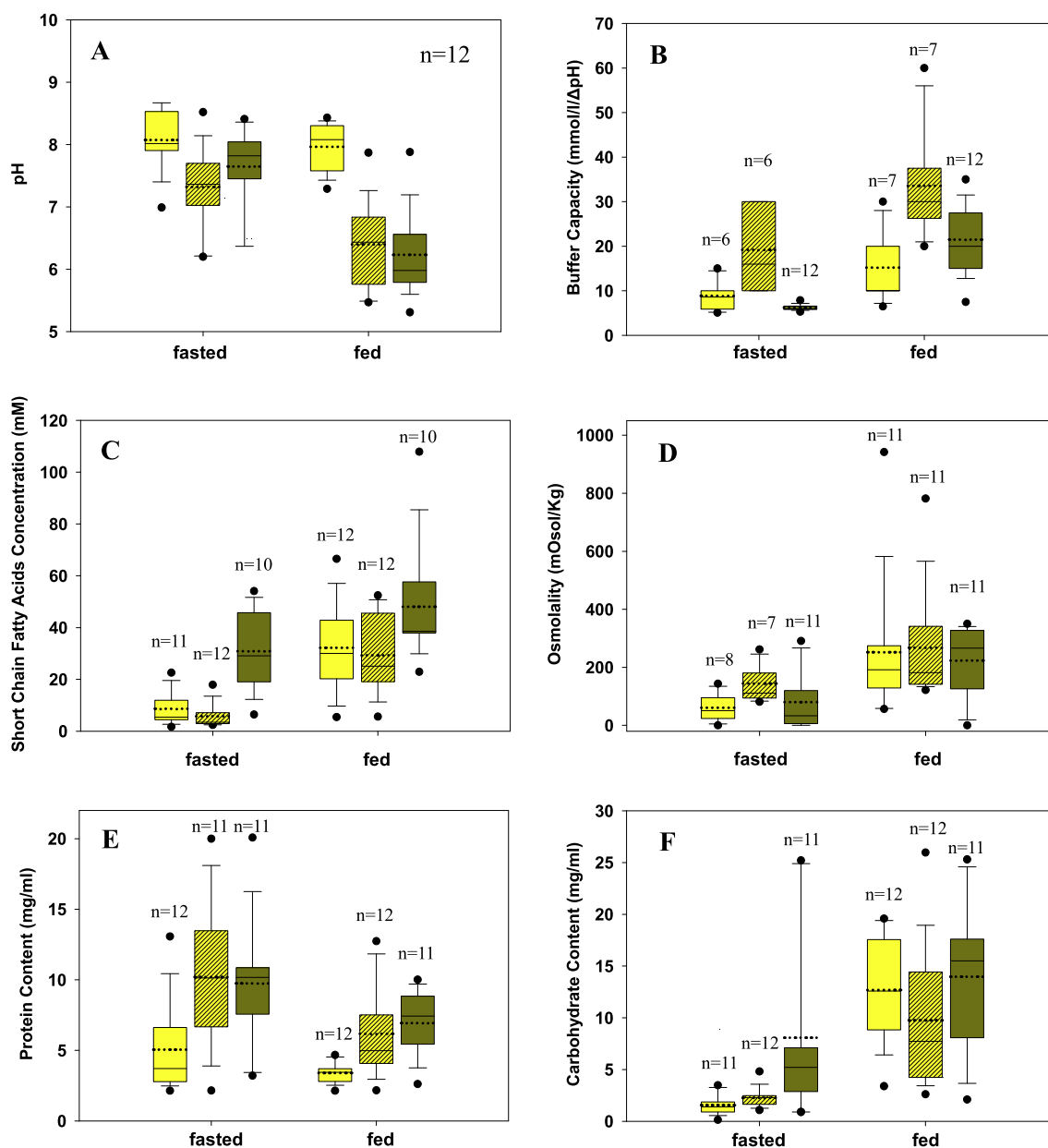


Fig. 4. pH, buffer capacity, short chain fatty acids concentration, osmolality, protein content and carbohydrate content of the contents of distal ileum (plain light-yellow boxes), of cecum (lined light-yellow boxes) and of ascending colon (plain dark green boxes) of healthy volunteers measured in the fasted and in the fed state. *n* is the number of subjects contributed to the construction of box plots. For each box plot and from bottom to top continuous horizontal lines indicate the 10th, 25th, 50th (median), 75th and 90th percentile, black dots indicate the individual outlying data points, and dotted line indicates the mean value.

due to bile acid reabsorption in the distal ileum, bile acid concentrations in the lower intestine are significantly lower (mean values range from 70 to 500 μM) than in the upper small intestine, regardless of the prandial state (Pentafragka et al., 2019). Higher concentrations of primary bile acids (cholic acid, chenodeoxycholic acid) than secondary bile acids (deoxycholic acid, lithocholic acid) have been observed in the fed ascending colon, which is the opposite to the fasted state situation. Diakidou and colleagues concluded that that as more bile acids enter the colon after the consumption of a meal, the capacity for conversion to secondary bile acids is saturated, resulting in more bile acids in the primary form (Diakidou et al., 2009).

Caecal and ascending colon contents have almost twice the concentration of free fatty acids concentration than ileal contents in both prandial states. Concentration of total phospholipids (phosphatidylcholine and lyso-phosphatidylcholine) in distal ileum did not differ significantly in the fasted state compared to the fed state. In the fasted

state, total concentration of phospholipids is increased significantly from distal ileum to cecum and then to the ascending colon. In the fed state, there is no difference in phospholipids between distal ileum and cecum but there is an increase in the ascending colon. Cholesterol levels do not differ within each region of the lower intestine with the dosing conditions (Diakidou et al., 2009; Reppas et al., 2015; Pentafragka et al., 2019).

4.2. Availability and distribution of fluids in the human GI tract

In terms of oral drug delivery, luminal fluids have two major functions. Firstly, they are needed to dissolve the drug as it is widely accepted that only drug in solution can be absorbed. Secondly, luminal fluids enable drug absorption by bringing the dissolved drug into contact with the absorptive tissues of the GI tract and by acting as a carrier during the uptake into systemic circulation. Hence, the knowledge of

the availability and distribution of luminal fluids is a prerequisite for successful oral drug delivery. However, it must be kept in mind that the fluid volumes in all parts of the GI tract are highly dynamic and the result of an interplay between secretion, absorption as well as ante-grade and retrograde transfer of contents. In general, the human GI tract handles 8–10 L of fluids each day. From this volume, only about 2–3 L arise from the intake of food (20–30%) and drinks (70–80%). The major part is due to the secretion of digestive juices. Most of this water is absorbed in the upper intestine, but each day around 1.5 L of fluid arrive in the colon. Only about 200 mL of water are excreted via the feces (Jéquier and Constant, 2010; Ritz and Berrut, 2005). This daily fluid turn-over in the human GI tract certainly affects drug absorption and therefore, the availability and distribution of fluids within stomach, small intestine and colon as well as their implications for oral drug absorption shall be described in the following sections. Particular attention will be paid to the dynamic changes of fluid volumes in the different parts of the GI tract.

4.2.1. Stomach

The storage of incoming food as well as its enzymatical, chemical and physical processing are the main functions of the stomach. Simultaneously, it delivers small portions of pre-digested contents, the so-called chyme, to the small intestine, where nutrient and drug absorption can take place. Thus, the volume of gastric contents is the result of the volume of incoming food and liquids, the volume of oral and gastric secretions minus the volume of contents emptied into the small intestine. To the best of our knowledge, absorption of water from the stomach can be neglected.

In the fasting state, the residual fluid volume is generally small with values below 100 mL as was shown in various MRI studies (Grimm et al., 2018a; Mudie et al., 2014). In a recent publication by Grimm and colleagues the interindividual range of residual volumes in the stomach after an overnight fast of at least 10 h was 17–93 mL with a mean of 49 ± 19 mL. The intraindividual range was comparable and amounted to 22–77 mL with a mean 44 ± 18 mL (Grimm et al., 2018a).

The fluid dynamics after intake of food and liquids depends mainly on the caloric value of the ingested contents. Whereas non-caloric fluids are emptied rapidly, the gastric emptying of caloric contents can take up to several hours, depending on further parameters such as food texture or caloric density. From a regulatory point of view, the intake of a drug on a fasted stomach is defined as the intake of the drug together with 240 mL water after a fasting period of minimum 8 h. The resulting gastric fluid volumes over time were studied recently by Mudie and colleagues with the aid of magnetic resonance imaging (MRI). It can be seen from Fig. 5 that the initial fluid volume measured 2 min after fluid intake reaches a level of 242 ± 9 mL. Since this volume is already lower than the sum of the ingested water and the residual gastric fluid, which would be 275 mL, it can be assumed that gastric emptying of non-caloric fluids starts almost immediately. Subsequently, gastric emptying follows 1st order kinetics and after around 30–45 min the gastric fluid volume typically returns to levels measured prior to water intake (Mudie et al., 2014).

On the other hand, the intake of food can cause elevated gastric content volumes (GCV) for several hours, which can have dramatic consequences for the gastric residence times of oral drug products as well as drug release from these products. It was recently shown that after the ingestion of the high-caloric (800–1000 kcal), high-fat standard breakfast, as recommended by FDA and EMA for studies, in which the food effect on oral bioavailability or bioequivalence in the fed state is tested, the GCV rises from 4 to 65 mL in fasted state to 500–628 mL directly after meal intake (Fig. 6) (Koziolek et al., 2014a, 2014b). This volume is the sum of the volumes of the meal, residual gastric contents as well as oral (e.g. saliva) and gastric secretions. The contribution of digestive juices to fluid volume in the stomach should not be underestimated. Whereas unstimulated salivary flow rates are in the range of 0.1–0.5 mL/min, stimulated salivary flow can be up to 10 mL/min. As

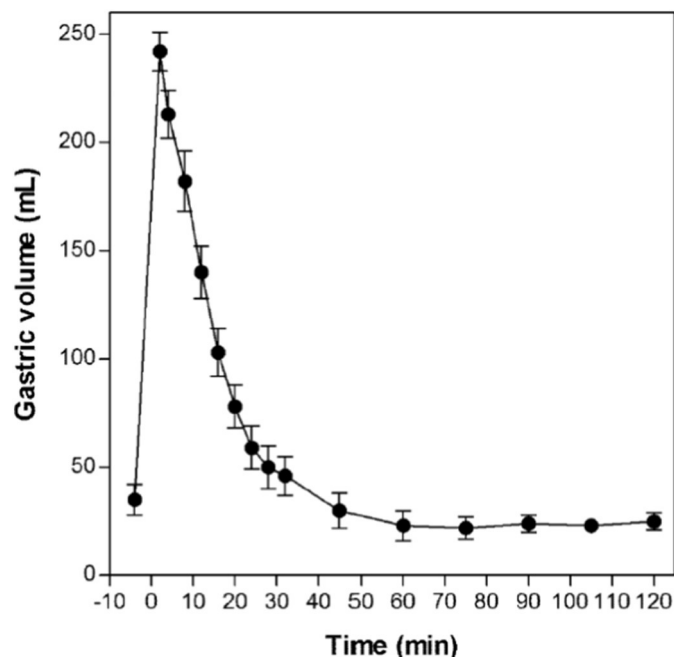


Fig. 5. Mean gastric volume \pm SEM before and after the intake of 240 mL of water at $t = 0$ min in 12 healthy subjects. Reprinted from Mudie et al., 2014, with permission from the ACS. Further permissions related to the material excerpted should be directed to the ACS.

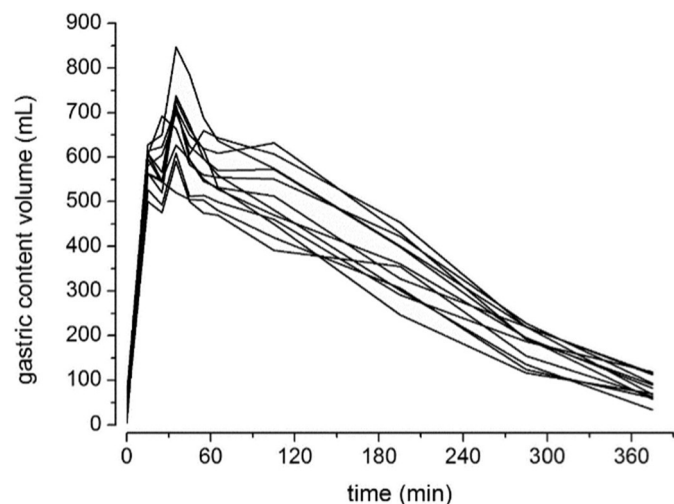


Fig. 6. Individual gastric content volumes (mL) over time (min) determined before and after administration of the high-caloric FDA breakfast in 12 healthy volunteers by using MRI. The gastric content volume at 0 min represents the residual gastric content volume after an overnight fast of at least 10 h. All subjects consumed the entire meal within 15 min. After 30 min, the subjects drank 240 mL of water inside the MRI scanner in order to simulate the intake of water during drug administration. Adapted from Koziolek et al. (Koziolek et al., 2014a, 2014b).

the majority of this volume is swallowed, oral secretions account for a considerable part of the total gastric volume. Gastric secretions are also stimulated by meal intake in order to allow sufficient digestion of the food. In the fasted state, gastric secretion rates of 1 mL/min or lower are described in literature (Dubois et al., 1977), whereas after consumption of solid foods initial gastric secretion rates of 10 mL/min and higher can be measured (Malagelada et al., 1976). In an MRI study published by Sauter and colleagues, approximately 50% of the gastric content volume present 100 min after intake of 400 mL of a viscous chocolate

drink was due to gastric secretions (Sauter et al., 2011). It can be seen from Fig. 6 that the GCV is fairly constant for the first 60–90 min, which may be explained by the circumstance that secretion and emptying are in balance. Interestingly, during this time the intake of 240 mL of water (after 30 min) only causes a minor peak in GCV that disappears quickly within 15–35 min. This distinctive profile is the result of the Magenstrasse (stomach road), a mechanism that allows the fast emptying of liquids from the fed stomach (Koziolek et al., 2014a, 2014b). In a follow-up study, the presence of the Magenstrasse was also confirmed for other foods that differed in texture and caloric density (Grimm et al., 2017). With respect to oral drug delivery, this mechanism is highly relevant as it allows the early emptying of drug into the small intestine and thus, the early onset of systemic drug concentrations, given disintegration of the dosage form and drug dissolution are fast as well. Recently, Sager and colleagues have developed a simple and reliable technique to describe gastric emptying of water in both fasted and fed state. This method is based on the quantification of caffeine in human saliva and can be easily combined with conventional pharmacokinetic studies (Sager et al., 2018). By this, the contribution of gastric emptying to the onset of systemic drug concentrations can be evaluated.

In contrast to the emptying of liquids, the gastric emptying of solid foods can be typically described by zero order kinetics. As a rule of thumb, the gastric emptying rate of caloric contents typically amounts to 2–4 kcal/min. In case of the FDA standard breakfast, the GCV decreases with a relatively constant rate of 1.7 ± 0.3 mL/min. Thus, even > 6 h after meal intake the GCV is still elevated compared to the GCV measured prior to meal intake. However, by this time the meal will be highly diluted owing to ongoing secretion and emptying. In any case, it should be noted that mixing in the stomach is not as vigorous as generally assumed. Marciani and co-workers nicely illustrated by MRI that dilution of a viscous locust bean gum meal with gastric secretions is slow (Marciani et al., 2001). Unfortunately, little is known about the fluid dynamics in the human stomach since the *in vivo* imaging of these processes is complex. Nonetheless, *in silico* simulations based on computational fluid dynamics by Ferrua and Singh suggest that peristaltic waves cause higher shear rates in the antrum, which facilitates mixing of gastric contents with secretions (Ferrua et al., 2014; Ferrua and Singh, 2010). In contrast, in the fundus mixing is generally poor. Thus, a dosage form located in the fundus will be exposed to small shear stresses and low shear rates, whereas in the antrum, shear rates and stresses are higher. This may have dramatic consequences for disintegration of dosage forms as well as the process of dissolution. Longer localization within the fundus can be expected to be responsible for the lag times often seen after drug administration in the fed state (Koziolek et al., 2016).

4.2.2. Upper intestine

As mentioned earlier, the availability of fluids within the small intestine is a prerequisite for drug absorption as fluids play a major role in dissolution, distribution of the drug within the intestinal lumen and absorption. In particular, for poorly water-soluble drugs administered at higher doses the dissolution of the drug in intestinal fluids may be problematic. On the other hand, dissolution in small volumes may lead to high concentrations thereby facilitating processes including passive diffusion and/or saturating efflux transporters.

The availability and distribution of fluids within the small intestine was largely unknown, but novel imaging techniques such as magnetic resonance imaging were successfully applied in the recent fifteen years to shed light on this important aspect of oral drug delivery. Schiller and co-workers were among the first to apply MRI in order to measure the fluid volumes in the small intestine in fasted and fed state (Schiller et al., 2005). They demonstrated that the small intestinal fluid volume in the fasted state can be highly variable and ranging from 45 to 319 mL. On average, the small intestinal fluid volume was measured as 105 ± 72 mL. However, this volume is distributed over the entire length of the small intestine in several “fluid pockets”. The largest fluid

volume is typically present in the terminal ileum, because contents accumulate at the ileocaecal junction before they are transferred into the caecum. One hour after the intake of a high-caloric breakfast, the fluid volume amounted to just 54 ± 41 mL, which was significantly lower than the fasted state volume. Again, the distribution of fluid in various “fluid pockets” was observed. In a later study, the changes of intestinal fluid volumes were investigated over a period of > 8 h by Marciani and co-workers. They also found a decreased fluid volume after 1 h, but on further study, they revealed that the fluid volume begins to increase after around 90 min and a maximum fluid volume is reached after approximately 3 h. At this time, the volume is slightly higher compared to fasted state values (Marciani et al., 2010).

Several MRI studies have also looked at the contribution of certain nutrients on small intestinal fluid volumes. Grimm and colleagues have shown that small intestinal fluid volume after intake of grapefruit juice is elevated for longer times compared to the intake of the same volume of water. Whereas initially the small intestinal fluid volumes are comparable, the fluid volume 80 min after drinking was twice as high as for the grapefruit juice compared to water (Grimm et al., 2018b). The authors assumed that this can alter drug absorption of certain drugs. The different volumes can be explained by the presence of fructose, which is absorbed slowly and thus causes water influx into the intestinal lumen due to osmotic effects (Murray et al., 2014).

Groups have also tried to determine the rate of water absorption from the small intestine by using D₂O as a marker. Péronnet and colleagues obtained a rate of 3–3.5 mL/min for intestinal water absorption after intake of 300 mL of water by pharmacokinetic modeling (Péronnet et al., 2012). However, other groups determined rates of up to 25 mL/min with significant regional differences. Lambert and colleagues demonstrated that water absorption in the proximal intestine is higher compared to distal parts (Lambert et al., 1997). The net water flux across the intestinal membrane is further affected by secretion of fluids and is thus affected by the osmolality and the electrolyte and nutrient (e.g. carbohydrates) composition of the intestinal contents (Shi and Passe, 2010).

In general, it should be considered that the fluid turn-over is highly dynamic. Secretion, absorption as well as transport of fluids in antegrade and retrograde directions all occur at the same time. Unfortunately, the kinetics of these processes are still largely unknown and therefore, it is almost impossible to assess the role of fluid in oral drug delivery. We have noted in past MRI studies that fluid present in the small intestine can disappear almost instantaneously from the lumen, but re-appear in another moment. Whilst fluid flow across the mucosa is not fully understood and more or less unpredictable, pharmaceutical scientists should be careful in using intestinal fluid volumes for *in vitro* and *in silico* simulations.

4.2.3. Lower intestine

Roughly 1–2 L of intestinal contents are transferred into the large intestine every day, from which around 200 mL are excreted via the feces each day. During colonic transit, the viscosity of the contents increases, whereas the volume of free fluid decreases. Hence, the conditions for drug dissolution in the colon are relatively poor, which should be particularly concern in the development of colon-targeted dosage forms.

The MRI study by Schiller and colleagues revealed that the luminal fluid volume in the colon in fasted state is relatively small with measured values in the range of 1–44 mL and a mean of 13 ± 12 mL. This fluid is found primarily in several fluid pockets located in the ascending and descending colon. After meal intake, the colonic fluid volume slightly increases to values of 2–97 mL with a mean of 11 ± 26 mL. The number of fluid pockets was also higher in the fed state (Schiller et al., 2005). In a later study, Murray and co-workers investigated the fluid volume in the colon and the number of fluid pockets in fasted state (Murray et al., 2017). After an overnight fast of at least 10 h, the subjects drank 240 mL of water. Subsequently, the volumes of free fluid

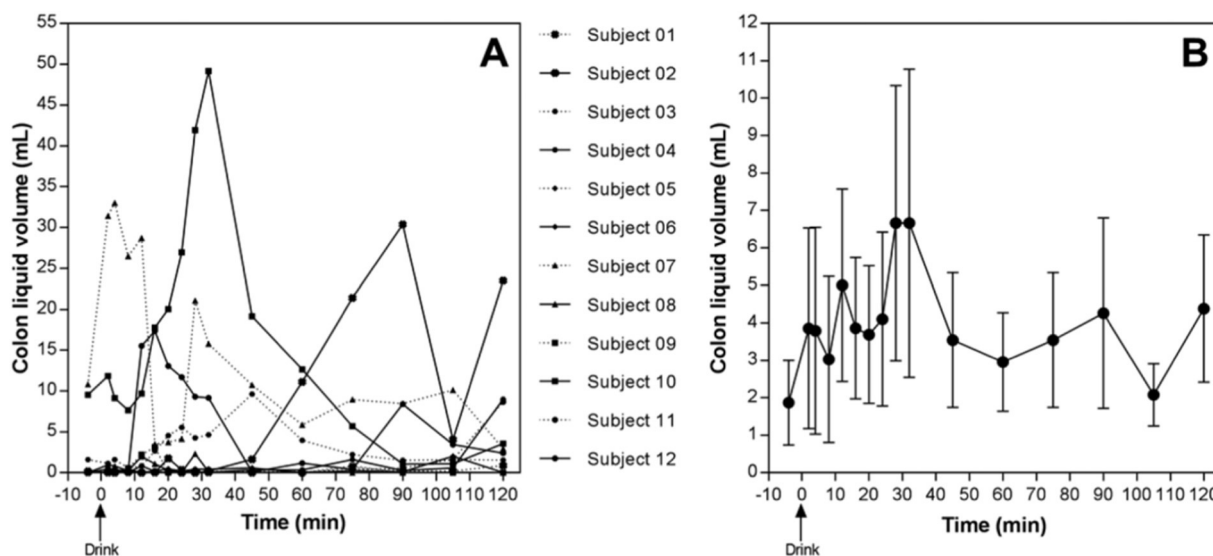


Fig. 7. Individual (A) as well as mean \pm SEM (B) colonic liquid volumes over time as determined by MRI in 12 healthy subjects. At $t = 0$ min all volunteers drank 240 mL of water on a fasted stomach. Reprinted from Murray et al., 2017 (<https://pubs.acs.org/doi/10.1021/acs.molpharmaceut.7b00095>), with permission from the ACS. Further permissions related to the material excerpted should be directed to the ACS.

were measured over a time course of 2 h. Fig. 7 illustrates that the liquid volume reached its maximum 30 min after fluid intake, and again shows the large variability of colonic fluid volumes. Moreover, free liquid was found to be mainly present in the ascending colon. The data presented in this work are useful for the characterization of the conditions in bioequivalence and bioavailability studies: The observed increase in colonic fluids probably resulted from the gastroileal reflex, and it is unlikely to be the fluid that was recently ingested.

In an aspiration study, Reppas and colleagues were able to collect 5.0 ± 2.1 mL from the fasted caecum and 8.0 ± 2.7 mL from the fed caecum (Reppas et al., 2015). In an earlier study, the same group collected contents from the ascending colon with the aid of a colonoscopy over a period of approximately 10 min (Diakidou et al., 2009). In this study, the volume of the contents in the ascending colon was 22.3 ± 7.7 mL in the fasted state and 29.9 ± 10.8 mL in the fed state. Interestingly, the aqueous fraction was higher in fasted state ($70.3 \pm 17.0\%$) as compared to the fed state ($56.0 \pm 9.0\%$). These values correlate well with the MRI data described above.

It should be noted that the availability of fluids for dissolution and absorption also depends on colonic motility, since it affects dosage form transit through the colon as well as the transit of luminal contents. Only dosage forms that are in contact with fluid are able to release the drug in a relevant manner. However, drug absorption is typically limited to proximal parts of the colon (Wilson, 2010).

The review to this point has considered fluids and an understanding of some of the microstructural components of the gut wall. Fluid, food and drug forms are propelled by mechanical forces in the gut. In the clinic, the applications of imaging have been especially important in the diagnosis of disorders of gastrointestinal transit. These technologies have been adopted in pharmaceutical sciences to examine regional transit-absorption relationships.

5. Human studies to evaluate regional drug absorption

5.1. Imaging

Modern imaging techniques offer some options to study the fate of orally administered drugs and the influence of regional differences in GI tract. Mostly, the position of a drug delivery system in the GI tract can be more reliably determined using imaging techniques than with other methodologies like for example electronic delivery capsules equipped

with pH sensors, since even strong pH changes are not necessarily correlated with gastrointestinal transport events (Koziolek et al., 2014a, 2015; Schneider et al., 2016).

The different imaging methods have several distinct areas of application, as for example the tracking of dosage forms, the determination of dosage form, disintegration and dissolution, as well as the evaluation of luminal fluid volumes and their transfer. Furthermore, imaging techniques are utilized as an aid for correct gastrointestinal placing of intraluminal tubes. In these cases, Fluoroscopy is usually applied in order to verify the correct placement of catheters in the GI tract (Van Den Abeele et al., 2016; Yu et al., 2017). Moreover, the tracking of electronic capsules with programmable release (see Section 5.3.1.) via imaging techniques has also been used to verify their localization for site specific release, absorption or sampling purposes (Clear et al., 2001; Martin et al., 2003).

The in vivo visualization of molecules in humans is established for the investigation of the distribution and elimination of labelled drug substances such as ^{18}F -fluorouracil (Saleem et al., 2000), or ^{212}Pb -TCMC-trastuzumab (Meredith et al., 2014). However, the spatial and temporal resolution as well as the detection sensitivity of common imaging techniques is not high enough to visualize directly the transport of (even labelled) molecules through the intestinal wall. In addition, regulatory issues with such labelled drug substances often hinder human application. Therefore, for the evaluation of site-specific drug release or absorption, a combination of the simultaneous determination of drug absorption via pharmacokinetic measures and imaging of the location of the usually labelled drug or drug delivery system is applied. Thus, one main utilization of imaging techniques is the evaluation of transit conditions and site-specific release of dosage forms. Unfortunately, only a few sets of imaging data with focus on site specific absorption are available, although it well known that dosage form transit can massively influence subsequent absorption and exposure as shown for several drugs (Varum et al., 2010).

It should be appreciated that the gastric fate of a dosage form and the region-specific circumstances in the stomach are likely to cause differences in subsequent absorption. In that regard, gastric emptying and transit has extensively been studied using several imaging techniques in the fasted and the fed states, as detailed in Section 4.2 (Jones et al., 2012; Koziolek et al., 2014b; Mudie et al., 2014; Weitschies and Wilson, 2011). From imaging studies, there is no evidence for relevant drug absorption in the human stomach as gastric emptying correlates

extremely well even in case of highly absorbable drugs like caffeine and paracetamol (Paulick and Siegmund, 2013; Sager et al., 2018). Thus, drug absorption in humans is predominant in the small intestine or sometimes also the colon whilst gastric emptying often determines the absorption rate (Koziolek et al., 2016; Van Den Abeele et al., 2017a).

5.1.1. Results from human studies

The site specific disintegration and release of dosage forms can be studied in humans with several techniques including scintigraphy, magnetic resonance imaging (MRI), magnetic marker monitoring (MMM), alternating current biosusceptometry (ACB), radiography or fluoroscopy (Hunter and Taljanovic, 2003; Klausner et al., 2003; Sjögren et al., 2014; Weitschies and Wilson, 2011; Wilding et al., 2001). The most commonly reported approach to evaluate site specific absorption with imaging techniques is the combination of scintigraphy and pharmacokinetics, also referred as pharmacoscintigraphy (Srivastava et al., 2014). It requires the inclusion of a gamma-emitting radiopharmaceutical in the delivery system. The suitable tracer can be incorporated during the formulation of the dosage form, which is often a challenge with respect to the half-lives of the radioisotopes, or subsequently via a “drill and fill” procedure which is also common for labeling with ferromagnetic material as required for MMM. In this case a hole is drilled into the already produced dosage form (typically a tablet), the labeling material is included and the hole is sealed with a suitable substance afterwards. Sometimes the label can also be attached to the surface of a dosage form. This is most useful for oesophageal transit studies since the disturbed surface is not in contact with the mucosa.

A special approach is the combination of scintigraphic tracking of a programmable or remote-controlled telemetric device such as the IntelliSite® capsule and pharmacokinetics. In this case, a marker substance of choice for scintigraphic detection can be included into the drug reservoir. Several insights have been obtained using this approach. For example, a study using an investigational capsule which releases furosemide and theophylline on firing release in the gut showed a poor absorption of furosemide and theophylline from the colon, and also a much slower release and distribution due to the highly viscous colonic contents (Clear et al., 2001). It was also shown that leuprolide acetate is nearly not absorbed from the ileum and the colon (Doll et al., 1997) and that ranitidine is absorbed from the jejunum and the ileum to the same extent and rate (Pithavala et al., 1998). Using a radiolabeled electronic delivery capsule, it was observed that lumiracoxib is well absorbable from the proximal and the distal small bowel as well as from the colon (Wilding et al., 2004). The evaluation of tacrolimus also showed absorption from small intestine and colon (Tsunashima et al., 2014). In all of these studies, GI transit was evaluated in real time by gamma scintigraphy and release was triggered by remote control. Studies with labelled dosage forms have been performed to study regional drug absorption. Extended release morphine tablets with differing release characteristics were investigated and absorption of morphine was observed throughout the entire GI tract, although the release was reduced in distal parts (Olsson et al., 1995).

The investigation of diltiazem dosed as extended release pellets or as a solution in fasted and fed state showed that diltiazem was rapidly absorbed in the upper GI tract from the solution whilst diltiazem absorption from the pellet formulations continued for > 12 h, irrespective of fasted or fed state. No relevant differences in the PK profiles were observed, although the pellets had already arrived the colon after fasted administration at these late time points. Thus it seems that diltiazem can be absorbed from the colon to the same extent like from the small bowel and altered media did not affect the PK (Wilding et al., 1991). This was also observed with modified release mini tablets containing diltiazem (Wilding et al., 1995).

For other drug substances a high impact of gastrointestinal transit has been observed, for example in case of saquinavir administered as capsules in the fed or fasted state. In this case it could be demonstrated

that saquinavir is best absorbed from the upper small intestines, since emptying of intact capsules from the stomach after intake under fasting conditions resulted in a passage of unreleased substance through the regions with high absorption capacity and thus a reduced bioavailability. In contrast, the slow gastric emptying of dispersed saquinavir after capsule disintegration in fed stomach led to a higher absorption in the upper small intestinal region (Kenyon et al., 1998).

The concept of insufficient contact time to regions where absorption takes place is well established and has been addressed in the transit parameters including MTT (mean transit time), MRT (mean residence time), and MAT (mean absorption time) (Darwich et al., 2016). This was nicely demonstrated for gefitinib. Subjects with faster gastrointestinal transit showed a reduced bioavailability which was attributed to slow absorption from the upper GI tract and insufficient absorption in more distal regions (Wilson et al., 2009).

Colon targeting of locally acting substances like mesalazine (5-ASA) is another field for the application of imaging methods. For example, for different time controlled formulations of mesalazine aimed for colon targeting, it could be demonstrated that mesalazine absorption correlated with time and site of disintegration (Steed et al., 1997). In a study with 4-aminosalicylic acid (4-ASA) it could be shown that only the metabolite was found in plasma if the disintegration took place in colon (Tuleu et al., 2002).

In case of ziprasidone, low colonic absorption has been observed (Thombre et al., 2015). In contrast, scintigraphic studies with modified release formulations of tamsulosin, dexamethasone or ibuprofen showed good absorption from colon for these drugs (Hodges et al., 2013; Kenyon et al., 1997; Wilson et al., 1989).

In addition to the scintigraphic investigations, it became also feasible to use MRI to study gastrointestinal transit, at least for single unit dosage forms. For example, ferromagnetic iron oxides can be used to visualize the location but probably not the disintegration of dosage form. Therefore, a combination with an absorbable marker substance or the drug substance of interest was required. Although there are several studies concerning the tracking of dosage form (Chaddock et al., 2014; Hahn et al., 2011; Kagan et al., 2006; Steingoetter et al., 2003), this method was typically not used for evaluation of site specific absorption but rather site specific disintegration (Grimm et al., 2019).

5.1.2. Pitfalls

Although imaging is usually performed to verify the localization in the GI tract, this issue may not optimally addressed with most of the applied imaging techniques so far described. In common gamma scintigraphy, a radiopharmaceutical is detected in 2-dimensional imaging therefore, the exact localization in GI tract could be doubtful in some investigations since the regional location lacks the anatomical references. This may lead to some complications regarding estimation of the position of the dosage form in the GI tract, since gastrointestinal anatomy differs even between healthy subjects (Srivastava et al., 2014). Comparable problems account for MMM and other techniques which also require anatomical location.

When using scintigraphy, this can be partially overcome by labelling of co-administered water with [^{99m}Tc]-DTPA chelate (Wilson et al., 2009) or of food labelled with ^{99m}Tc sulphur colloid which roughly delineates anatomical contours of the GI lumen (Cole et al., 2004). In addition, a commonly used approach is to use thoracic front and back “anatomical locators” by the attachment of one or more closed point sources with the radionuclide being measured affixed to the subject. Subsequent analysis uses acetate maps to locate gastric and colonic positions revealed by dispersion or second label to these landmarks (remembering to physically mark the skin!). Since a considerable interindividual variability in anatomical orientation and morphology has been reported by several workgroups, this might not be sufficient for a very specific and narrow definition of location in the GI tract (Sandberg et al., 2015; Steingoetter, 2015).

These problems can be avoided using MRI as it provides excellent



Fig. 8. Variability of intestinal anatomy within three subjects in T1w MRI.

anatomical images allowing the detection of the interindividual variability in GI anatomy as illustrated in Fig. 8.

5.2. Intraluminal sampling

Although the influence of GI physiology on intestinal drug disposition is extensive, the underlying mechanisms often remain difficult to identify. Indirect methods including deconvolution and PBPK modeling do not always capture the level of complexity involving a physicochemical event interacting in a variable physiological environment. To assist in understanding drug and formulation behaviour, direct measurement of intraluminal drug concentrations through local sampling allows integration of all the cogent data. Independent of regional differences, GI absorption is driven by local drug concentrations.

Intraluminal sampling enables the determination of drug

concentrations at the site of interest (stomach or intestine). When performing gastric and intestinal drug disposition studies, one (Fig. 9A) or more catheters (Fig. 9B) are introduced through the mouth or nose of a human volunteer. Catheters are positioned under fluoroscopic guidance and can be led through the pylorus to reach the small intestine (Brouwers et al., 2007). A schematic overview is provided in Fig. 9. Following correct positioning, volunteers can ingest a drug formulation, sometimes in combination with a solid or liquid meal (Brouwers et al., 2007). Gastric and intestinal fluids can subsequently be retrieved as a function of time, resulting in a detailed GI drug concentration profile (Brouwers et al., 2005). Following aspiration, these fluids can also be analyzed for physicochemical properties, enzyme activity and/or bile salts (Brouwers et al., 2007; Walravens et al., 2011; Riethorst et al., 2016). It should be noted that studies using catheters placed inside the GI tract are inherently invasive in nature and can thus potentially affect

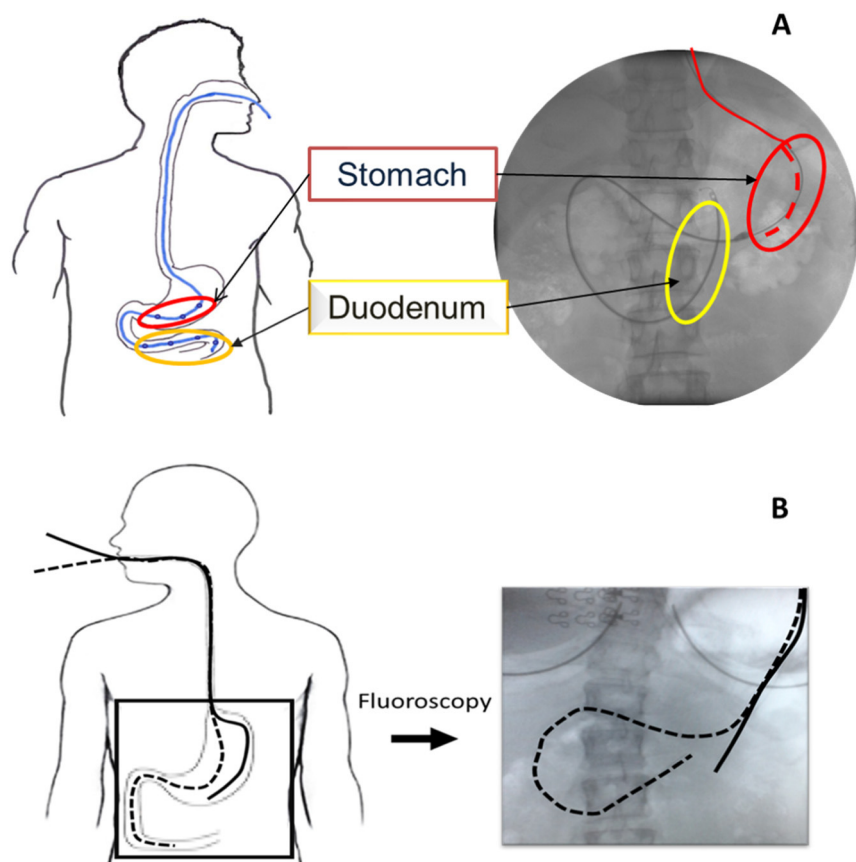


Fig. 9. Schematic overview of intraluminal sampling technique. Left: Catheter(s) are introduced through nose (A) and mouth (B) and positioned inside the stomach and the intestine. Right: X-ray images of (A) one catheter positioned inside the stomach and the intestine (A) and (B) a catheter positioned inside the stomach (solid line) and a catheter positioned inside the intestine (dotted line); catheters are traced for clarity.

normal gastrointestinal physiology. However, several studies indicate minor or non-significant effects of transpyloric tubes on gastric emptying, gastric secretions and duodenogastric reflux (Go et al., 1970; Longstreth et al., 1975; Näslund et al., 2000; Read et al., 1983; Rees et al., 1979).

Intraluminal sampling is often combined with concomitant blood sampling to relate gastrointestinal drug and formulation behavior to systemic drug disposition (Brouwers et al., 2007). In this case, as intraluminal sampling inevitably includes removing a certain amount of drug from the intraluminal environment, the volume of aspirated sample should be limited.

5.2.1. Gastric drug disposition studies

Various small-scale (typically 5 to 10 volunteers) clinical studies have been reported in which gastric drug disposition has been linked to systemic concentrations. For instance, Brouwers and colleagues monitored GI concentrations of fosamprenavir after oral ingestion of a commercial formulation (Telzir®). A food-induced delay in systemic amprenavir absorption was correlated to the postponed dissolution of the fosamprenavir tablet in the stomach as observed in the gastric concentration-time profile (Brouwers et al., 2007). Walravens and colleagues investigated the effect of pH and co-medication on the gastrointestinal absorption of posaconazole. Posaconazole was administered as a suspension to healthy volunteers with water or a cola beverage, with or without prior proton pump inhibitor administration. Although co-administration with a cola beverage did not alter the pH of the intraluminal environment compared to administration with water, it did increase posaconazole gastric concentrations which related to an increase in systemic exposure. Co-administration of esomeprazole led to an increased gastric pH, which was accompanied by decreased gastric and systemic exposure. Using intraluminal sampling, this study indicated that dissolution of posaconazole in the stomach dictates its systemic absorption (Walravens et al., 2011).

Gastric sampling studies can be expanded with simultaneous measurements of GI motility. Intraluminal pressure waves can be monitored in real-time using high resolution manometry (HRM). This technique uses a catheter with multiple pressure sensors along its length. Van Den Abeele et al. used this combined collection techniques to study the effect of sparkling water on the gastric behaviour of a paracetamol formulation (Dafalgan®) (Van Den Abeele et al., 2017b). In this study, volunteers were asked to ingest a paracetamol tablet with either tap water or sparkling water, after which gastric drug concentrations and gastric motility including other factors were monitored. When the formulation was ingested with sparkling water, faster drug absorption was observed which could be linked to faster intragastric tablet disintegration combined with increased gastric motility. A typical concentration drug profile of one volunteer from this study is given in Fig. 10. This investigation illustrates the potential intraluminal sampling can provide in capturing the interaction between GI physiology

and in vivo drug disposition.

5.2.2. Intestinal drug disposition studies

Besides gastric drug disposition, several studies have been published in which intestinal drug disposition was linked to systemic pharmacokinetics (Van Den Abeele et al., 2017c; Hens et al., 2016b; Hens et al., 2016a; Hens et al., 2015; Koenigsnecht et al., 2017). Van Den Abeele and colleagues monitored intestinal and systemic drug concentrations in healthy volunteers after oral intake of a commercial tablet of diclofenac (Cataflam®). A good correlation was found between the time needed to achieve maximal systemic drug concentrations (t_{max}) and maximal dissolved drug concentration in the duodenum (Fig. 11a) (Van Den Abeele et al., 2017c). Fig. 11b depicts the systemic concentration-time profile of a healthy volunteer.

5.2.3. In vivo permeability assessment

A more advanced application of intraluminal sampling is the Loci-I-Gut® Perfusion method. This method uses a multi-lumen catheter whose design includes two latex balloons that can be inflated to isolate a certain intestinal segment. This segment is subsequently perfused with a drug solution and the permeability is determined from the disappearance of drug during perfusion (Knutson et al., 1989). This method can also be applied to determine intestinal drug metabolism and luminal excretions (Petri et al., 2003) (Tannergren et al., 2003).

In the next section, the information on gastrointestinal transit gathered from indirect tests is considered. Such tests, which include breath tests, impedance monitoring of the stomach and ultrasonography will yield less information compared to more established digital imaging techniques but have special attributes for certain patient and volunteer groups.

5.3. Indirect sampling

5.3.1. Breath tests

In many gastrointestinal diseases, an alteration of gastrointestinal transit signals neuromuscular dysfunction, obstruction or an inflammatory condition and measurement of the pathophysiological changes useful in assisting interpretation and treatment. Early diagnostic studies of gut behaviour were confined to x-ray contrast tests using opaque markers or whole gut transit measurements using coloured beads (Wilson, 2010). Gamma scintigraphy of labelled meal components provided more representative regional gut transit information and therefore was more valuable. Scintigraphy is a gold standard for assessing changes in gastrointestinal transit as good anatomical resolution is not essential; however, it does expose the patient to low doses of radiation which is of concern where the very young or pregnant females are investigated. For several reasons therefore, a simpler technology may be useful provided that it has robust end points. In the case of the breath test, the concept was to use the

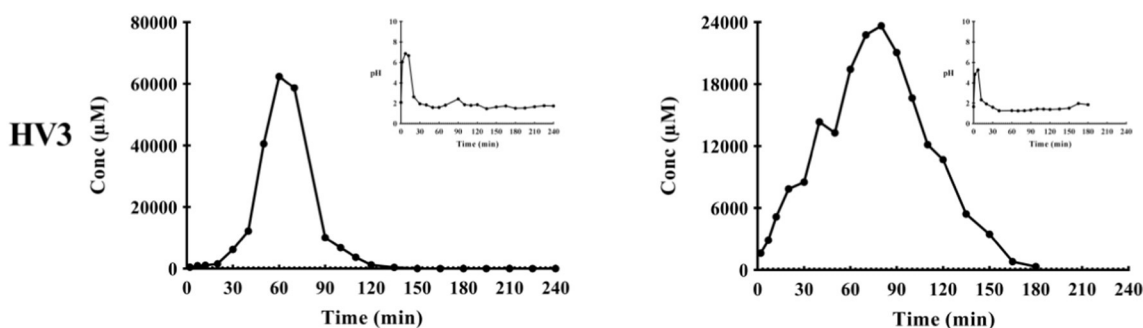


Fig. 10. Drug concentration determined in gastric fluids aspirated as a function of time from one healthy volunteer after intake of 1 tablet of Dafalgan® (500 mg paracetamol) with either 330 mL of tap water (left) or 330 mL of sparkling water (right). Inserts depict the pH of gastric aspirates as a function of time. Data adopted from Van Den Abeele et al. (2017b).

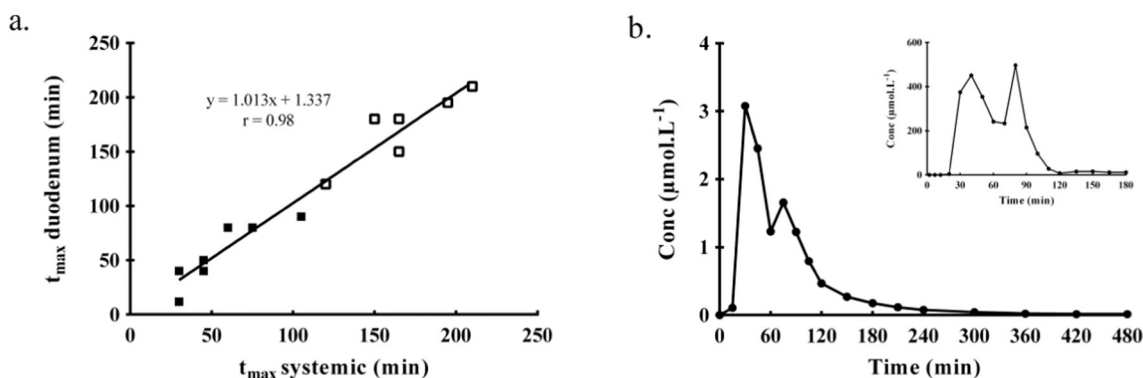


Fig. 11. (a) Correlation between time to maximal dissolved drug concentration in duodenum and time needed to achieve maximal systemic drug concentration ($r = 0.98$). (■) Fasted state data. (□) Fed state data. (b) Typical example of a second peak in the systemic concentration–time profile for one volunteer (fasted state). Insert depicts the corresponding dissolved drug concentration in the duodenum as a function of time. Data adopted from Van Den Abeele et al. (2017c).

bacterial fermentation of a carbohydrate to release hydrogen that would be exhaled.

The concept of using breath tests in metabolic studies had been stimulated by early studies of breath alcohol measurement described and used as the basis of patented instruments through 60's and 70's (Borkenstein and Smith, 1961; Borkenstein, 1971). Gas chromatography was then a new analytical instrument, essential in the oil and gas industry, which was beginning to be used for medical applications. Later applications have included detection of volatile components including alkanes in the breath of patients with lung cancer (Phillips et al., 2003). The fermentation of disaccharides and the production of the breath hydrogen was used to investigate lactose malabsorption (Newcomer et al., 1975). Hydrogen can be detected in the breath using gas chromatography or an electrochemical cell. This can be miniaturised to produce a portable, bedside instrument (Lee et al., 2000) but more recently telemetric capsules have been equipped with gas-sensing modality (Kalantar-Zadeh et al., 2018).

Since hydrogen can only be produced by bacterial reductive metabolism, this raised interest in the possibility of using the bacteria in the bowel to indicate the arrival of a test meal in the caecum, to determine the oro-caecal transit time (OCTT). It was important that the disaccharide was not absorbed during its transit through the small intestines so that the sharp rise in hydrogen in the breath signalled that the oro-caecal transit was complete. This remains a methodological difficulty. Rana and Malik summarized the use of hydrogen breath testing in three conditions: diagnosis of bacterial overgrowth of the small bowel (includes measurements using glucose); carbohydrate malabsorption (especially lactose or fructose) and thirdly altered transit of food through this the stomach and small intestines (Rana and Malik, 2014). In preparation for this test, the patient must not be on antibiotics, must refrain from consumption of any interfering slowly digested high-fibre food such as beans and cereals and must fast for 12 h prior to the test. They also must not smoke as carbon monoxide is picked up by the hydrogen detector, nor should they exercise before the test as this reduces breath hydrogen (Braden, 2009).

Measurements of oro-caecal transit time (OCTT) show some agreement with other measurement techniques although the endpoints can be difficult to note in some patients. It is generally shorter than that measured by scintigraphic techniques with normal values of around 90 min in controls (Rana et al., 2008), partly influenced by the presentation as a liquid. OCTT has been shown in other work to be delayed in type I and II diabetes (Rana et al., 2011; Faria et al., 2013). Hydrogen breath tests have also been used to measure OCTT during various stages of pregnancy, in tandem with ultrasound to measure gastric emptying (Chiloiri et al., 2001). In the ultrasound technique, the gastric volume is calculated from measurements of the longitudinal axis (L), and antero-posterior (AP) diameters (the cross-section is an ellipse) and applying the formula: $\text{volume} = \pi/4 \times L \times \text{AP}$.

Pregnancy is often accompanied by dyspepsia in the first trimester, which was noted in this study although the breath test indicated that gastric emptying was normal. In the third trimester OCTT was prolonged from 80 min (interquartile range 50–235.5 min) to 100 min (interquartile range 50.5–240 min), returning to 70 (interquartile range 40.5–240 min) in the post-partum period.

Unfortunately, there are individuals who do not have a bacterial population that can produce hydrogen and generate false negatives at a reported rate of 3–25% (Braden, 2009). These patients may be methane producers, since the hydrogen is directly incorporated in alkane formation. The alternative ^{13}C -breath test is a useful solution to the problem. Carbon 13 is a stable isotope and the additional neutron allows detection of the mass difference using a mass spectrometer. The natural abundance of carbon 13 is 1.11% of all carbon atoms and a wide range of substrates are available. This allows the exploration of liver and pancreatic function but ^{13}C -acetate has been widely used in OCTT measurements in children (Hauser et al., 2006) and ^{13}C -lactose ureide in veterinary OCTT studies (Wyse et al., 2004). Hauser and colleagues established the normal values for the gastric emptying of a standardized liquid meal in children between 4 and 15 years by combining scintigraphy using $^{99\text{m}}\text{Tc}$ technetium-labelled tin colloid with ^{13}C -acetate and found a strong correlation. Parker and colleagues have shown that the incorporation of breath tests markers into complex matrices generates many problems in data interpretation. The group compared the use of a ^{13}C -lipid constituents ^{13}C -octanoate (OCC), ^{13}C -acetate (ACC) and ^{13}C -trioctanoin (TCC) to measure the gastric emptying of lipid emulsions in healthy adults. The ^{13}C -acetate marker was used as a discriminatory signal since it partitions differently in fat compared to the two markers that should have produced similar results (OCC and TCC). They were unsuccessful due to the interaction of these markers with the lipid emulsion in the stomach environment, where protonation occurred and complications due to post-prandial processing of the TCC in the upper small intestine confounded interpretation (Parker et al., 2017). The breath test therefore remains of interest but use tends to be confined to use in groups that are unsuitable for more invasive tests or those involving radioactive tracers.

5.3.2. Electrical impedance monitoring and Electrogastrography (EGG)

Electrical resistance describes the process where a conductor resists any current going through opposing electrodes. As resistance decreases, the lower the voltage needed to sustain a given current flow. Electrical impedance is a related phenomenon but describes the resistance to change in voltage. In an electrical circuit, an inductor resists changes in the current whereas a capacitor resists changes (smooths out) the signal between electrodes when voltage is altered. From this it is seen that the basis of an instrument would be to measure the signal propagated across the electrodes placed opposite and around the circumference of signals propagated through the body section. It has long been realised

that this would be an attractive way to measure the changes in volume of an object in the body which had a material with a different dielectric; for example, the heart, the lungs and the bladder. By using multiple electrodes, a tomographic representation can be generated and this immediately suggested a possible use for measuring gastric emptying. Potentially such a system would be extremely cheap, portable and chemical free.

Chang and colleagues described a simple system to measure liquid gastric emptying in man which had a moderate sensitivity compared to scintigraphic measurement (Chang et al., 2001). One of the problems is that high amounts of body fat generates errors and in the Chang study, only 20/24 of the subjects could be used to generate successful measurements.

This issue obviously leads to clinical under-utilization of EGG and recently a group in Calgary described a modification to enhance the sensitivity of electrogastronomy, albeit at an early stage of development. In the study, the use of a miniaturised swallowable electronic oscillator in an expandable, self-disintegrating object (a pseudo bezoar) is described (Poscente and Mintchev, 2017). The requirements this test is that the unit is retained in the stomach for the duration of the test – essentially a gastroretentive device – which is then disaggregated to allow smooth movement out of the stomach at the end of the test. This might be aided by pH shift (administration of an antacid) or hot liquid to raise luminal temperature to 45 °C. The electronic oscillator sends out a single frequency signal which is easily discriminated background noise by cutaneous electrodes. The authors carried out a study in dogs before and after the administration of neostigmine to modify motility. Disintegration of the device was observed but the issue is that the battery remains intact which authors failed to comment upon.

The measurement of the action of cholinergic agents in pigs was described by a group in the Czech Republic (Kvetina et al., 2015) using a commercially available electrogastronomy system (Medical Measurement Systems B.V.) which is able to amplify and filter the signal from background interference of heart rhythms and breathing movements and ultraslow signal to preserve the dominant frequencies of the EGG signal. An array of seven electrodes to record the EGG and an abdominal belt to subtract the respiratory signal were positioned on the underside of the pig abdomen. The system was used by the authors to measure the effect of basic acetylcholinesterase modulators, atropine and neostigmine, on porcine EGG.

Overall, this technology has rather limited application in drug delivery and is likely to be supplanted by recent advances in point-of-care ultrasonography (PoCUS).

5.3.3. Ultrasonography

In real-time sonography (RTS), a closely coupled probe placed on the abdomen generates intermittent sound waves and receives back the echoes which are digitally processed to provide a black-and-white representation of the impedance that the wave encounters (see the excellent review by Abu-Zidan et al., 2011). Reflection from a solid object generates white pixels whereas in fluid filled compartments, the wave is propagated forward giving a black echogenic image. The frequency of the wave determines resolution and depth of penetration. The probe can also be run in continuous mode to generate Doppler images to study movement within the structure.

Gastric emptying can be measured by assembling cross sections across the organ when the stomach is full of liquid; however, if full of air, measurements become difficult. The location of the stomach when it is high in the abdomen, with the fundus obscured by the costal margin, can also frustrate measurement. For this reason, ultrasound measurements are often restricted to the antrum which is nearly always visible (Darwiche et al., 1999). Darwiche and colleagues used RTS to estimate the percentage of a 300 mL rice pudding meal in the stomach of normal controls and diabetic patients. At 15 min post-ingestion, diabetic patients emptied 29% of the test meal versus 63% for the controls.

Ultrasound has also been used to measure gastric emptying of water in pregnancy at full term (Wong et al., 2002). The risk of pulmonary aspiration of stomach contents during anaesthesia at delivery is well established but rare; it is however a defined risk and before elective induction; the physicians prefer the stomach to be empty. Antral cross section measurements were generated and the half-time of gastric emptying from both ultrasound measurements and serum acetaminophen concentrations after administration of 15 mL of a liquid acetaminophen preparation with 50 or 300 mL water. Data collected showed that healthy, full term women, not in labour had unaltered gastric emptying rates compared to controls.

Bataille and colleagues conducted an ultrasound examination in women during labour who were receiving epidural anaesthesia and showed that there was a slowing of emptying in early labour, suggesting that the risk of aspiration was supported (Bataille et al., 2014). Sumpelmann and colleagues have conducted an ultrasound assessment of healthy pre-school children. The objective here was to assess the feasibility of the ultrasound measurement to measure gastric emptying of light meals in order to generate protocols to decrease the risk of aspiration in anaesthesia but allow the children appropriate nutrition (Sumpelmann et al., 2017).

Critically ill patients may have an issue of retained gastric volume and ultrasound examination has been used to stratify those patients who are at risk. The measurement should be quick and simple and often must be completed at the bedside; the objective is to identify those patients whose gastric emptying is so slow that they require endotracheal intubation (Hamada et al., 2014). A patient with a full stomach that is emptying slowly with multiple co-morbidities is difficult to manage and is a leading cause of death in anaesthesia-associated aspiration. Sumpelmann and colleagues report on an ultrasound investigation of a 48y patient using a procedure that has found wide use clinically - PoCUS. Gastric point-of-care ultrasound. This was used to assess the emptying of the stomach following infusion of a prokinetic - erythromycin at 3 mg kg⁻¹ and confirmation of gastric emptying prior to surgery (Sebrechts et al., 2018).

Since 2010, ultrasound devices based on portable devices including attachments for mobile phones have interested researchers and health-conscious individuals. The fabrication of cheap robust capacitive transducers as an alternative to piezo-electric elements was described in 2018 (Gerardo et al., 2018) and is likely to result in a new generation of medical apps on smartphones. Very recently, the Butterfly IQ handheld ultrasound set has been launched after raising \$250 million from an investment syndicate. The probe connects to a phone or iPad as the reporting/recording device. These highly mobile instruments have an obvious place in medicine and medical journals are now reporting the deliberations of experts establishing recommendations for training and interpretation of data (Diprose et al., 2017; Cardim et al., 2018).

Finally, we consider methods to explore regional absorption and trek back towards an in vitro system that is able to capture mechanistic information that may be unavailable from clinical studies.

6. In vitro/in silico methods to evaluate regional drug absorption

Knowledge of regional changes in the properties of the gastrointestinal tract are an essential component of mechanistic models to predict drug absorption and are particularly important to guide development of controlled release dosage forms. Primary determinants of regional absorption are regional changes in solubility and permeability. The solubility of a drug in different portions of the gastrointestinal tract might vary due to changes in pH, buffer capacity, surfactant concentration, fluid volume and viscosity. However, the solubility changes are not common across all drugs but are dependent on specific drug properties. For example, the regional luminal solubility of the neutral molecule prednisolone, does not vary significantly while mesalamine, a zwitterion, shows increasing solubility down the GI tract. To guide development of a drug product, knowledge of regional variation in

solubility can be important but measurement in aspirated luminal fluids is not always convenient. In more practical terms, the use of biorelevant media simulating the *in vivo* situation such as FaSSGF and FeSSGF which simulate the fasted and fed state stomach respectively, while FaSSIF and FeSSIF media can be used for the upper small intestine (Jantratic et al., 2008). Different levels of biorelevant media have been defined with Level I media reflecting luminal pH and buffer capacity whereas Level II also take bile acid content, digestion products, and osmolality of luminal contents into account (Markopoulos et al., 2015a). In addition, media simulating human colonic fluids have been developed and were evaluated by measuring solubilities for ketoconazole, danazol and felodipine which was compared with measurements in sampled human colonic fluids (Vertzoni et al., 2010). Composition of media simulating the distal ileum (SIFileum), ascending colon in the fasted state (FaSSCoF) and in the fed state (FeSSCoF) have been defined (Vertzoni et al., 2010; Markopoulos et al., 2015b). These region specific biorelevant media are now standardized, commercially available and widely used across laboratories to generate data as input to physiologically based absorption models. However, there is still need for more verification of their utility to predict clinical performance with different drugs and formulations. Furthermore, more consideration of the time-dependency of solubilization is needed. This has already been initiated for media simulating conditions in the proximal GI tract (Jantratic et al., 2008) but obviously should apply to other regions as well.

Permeability is also a key determinant of regional absorption. The Ussing chamber is a well-established *in vitro* tool which has been applied to measure regional permeability in sections taken from duodenum, jejunum, ileum and colon which are mounted to allow access to acceptor and donor compartments. Overall highly permeable compounds show similar P_{app} in the colon as in the small intestinal segments, while a lower P_{app} in colon is seen for more polar compounds (Sjoberg et al., 2013; Haslam et al., 2011; Rozehnal et al., 2012). Transporter-mediated uptake or efflux as well as intestinal metabolism can also be demonstrated using tissue samples mounted in the Ussing chamber. Overall, the research on regional permeability changes still has many gaps to fill. Data on intestinal transporter protein expression has started to emerge (Harwood et al., 2015) and the technology for measurement advances rapidly (Nakamura et al., 2016). However, comparability of data from different laboratories still needs to be confirmed as does the successful integration of expression data into physiologically based models for prediction of the effect on drug regional absorption.

Aside from solubility and permeability, regional dependent tools to estimate dissolution and precipitation are important. Various *in vitro* tools to study dissolution in the stomach and upper small intestine have been described (Kourentas et al., 2016) and recently, a two-stage single-compartment *in vitro* dissolution test for distal ileum and proximal colon was proposed based on commercially available setups (Markopoulos et al., 2015b). Data generated in this system were input to a PBPK model and simulations were shown to agree with clinical data for two immediate release highly dosed compounds as well as for one colon targeting product (Markopoulos et al., 2015b; Georgaka et al., 2017). Tools for precipitation were recently reviewed (O'Dwyer et al., 2019).

Regional dependent solubility and permeability measurements along the length of the human gastrointestinal tract are challenging to obtain for most drugs and so there have been efforts to develop *in silico* models to predict these properties. For solubility, PBPK absorption models have implemented approaches which attempt to predict solubility throughout the GI tract based on a limited set of measured data and knowledge of the variation of luminal fluids (Parrott et al., 2009; Patel et al., 2014; Sjögren et al., 2013). Generally, these models estimate the effect of ionization on solubility based on a drug's pKa values and knowledge of variation in regional pH, while, for lipophilic molecules where bile salts are important for solubilization, models estimate regional solubility based on regional bile salt concentrations and a

compound specific bile salt solubilization ratio. This ratio is generally estimated based on the compound lipophilicity (Mithani et al., 1996) or from measurements made in media containing a known concentrations of bile salts.

Physiologically based absorption models have also implemented predictive models to estimate regional permeabilities. Typically, permeability is measured in an *in vitro* experiment and this value is then transformed to an estimate of *in vivo* jejunal permeability based on a correlation built for a set of reference drugs where permeability has been measured. Regional permeability changes may then be scaled based on knowledge of the changing surface area due to different gut dimensions, intestinal folding and the density of villi and microvilli (Olivares-Morales et al., 2015). Some *in silico* models also attempt to account for the changing mixture of transcellular and paracellular transport in different regions as well as the effect on permeability of varying drug ionization due to different regional pH. These more complex mechanistic models are challenging to parameterize and verify and some commercial PBPK tools include empirical Absorption Scale Factors optimized so that simulations best match measured human absorption data for a set of reference drugs (Parrott and Lave, 2010). Overall there is great potential for physiologically based models to leverage knowledge of regional absorption changes to optimize clinical formulation development. Achievement of this potential will require further research to develop predictive *in vitro* tools and importantly the verification that data generated with these tools translates to clinical performance.

7. Conclusions

The impact of regional differences in anatomy and physiology along the gastrointestinal tract on the absorption of drugs is profound and for some classes of active pharmaceutical ingredients, notably small orally-administered biologics such as desmopressin or cyclosporin, the challenge of understanding the nature of the barriers is daunting. In these investigations, the integration of knowledge requires an appreciation of events at the cellular level: examining the flux of drug molecules through the absorbing membranes of different regions of the gut with those aspects of formulation which are influenced by motility and interaction with food at the macro scale. Pharmaceutical scientists are great 'adopters' of clinical and chemical technology and progress in optimisation of dosage form has borrowed heavily from clinical imaging disciplines as noted in this review. A next generation of imaging devices and technologies based on smart-phone add-ons might lead us to new possibilities for looking at regional drug absorption in special groups, within their home locations, including the young and the elderly.

Acknowledgments

This work was supported by a European Cooperation in Science and Technology Action [UNGAPE (CA16205)]. Christina Pentafragka was supported by a European Union, Horizon 2020 programme, under grant agreement No. 674909 (PEARL).

References

- Abrahamsson, B., Pal, A., Sjöberg, M., Carlsson, M., Laurell, E., Brasseur, J.G., 2005. A novel *in vitro* and numerical analysis of shear-induced drug release from extended-release tablets in the fed stomach. *Pharm. Res.* 22, 1215–1226. <https://doi.org/10.1007/s11095-005-5272-x>.
- Abu-Zidan, F.M., Hefny, A.F., Corr, P., 2011. Clinical ultrasound physics. *Journal of Emergencies, Trauma and Shock* 4 (4), 501.
- Al-Marhoon, M.S., Nunn, S., Soames, R.W., 2005. Effects of cagA+ and cagA- strains of *Helicobacter pylori* on the human gastric mucus layer thickness. *J. Gastroenterol. Hepatol.* 20, 1246–1252. <https://doi.org/10.1111/j.1440-1746.2005.03853.x>.
- Andrianifahanana, M., Moniaux, N., Batra, S.K., 2006. Regulation of Mucin Expression: Mechanistic Aspects and Implications for Cancer and Inflammatory Diseases. <https://doi.org/10.1016/j.bbcan.2006.01.002>.

- Atuma, C., Strugala, V., Allen, A., Holm, L., 2001. The adherent gastrointestinal mucus gel layer: thickness and physical state in vivo. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 280 (5), G922–G929.
- Ayabe, T., et al., 2000. Secretion of microbicidal α -defensins by intestinal Paneth cells in response to bacteria. Available at: <http://immunol.nature.com>, Accessed date: 24 August 2018.
- Bajka, B.H., et al., 2015. The influence of small intestinal mucus structure on particle transport ex vivo. *Colloids Surf. B: Biointerfaces* 135, 73–80. <https://doi.org/10.1016/J.COLSURFB.2015.07.038>. Elsevier.
- Barker, N., et al., 2007. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* 449 (7165), 1003–1007. <https://doi.org/10.1038/nature06196>. Nature Publishing Group.
- Barlow, N.E., Bolognesi, G., Haylock, S., Flemming, A.J., Brooks, N.J., Barter, L.M., Ces, O., 2017. Rheological droplet interface bilayers (rheo-DIBs): probing the unstirred water layer effect on membrane permeability via spinning disk induced shear stress. *Sci. Rep.* 7 (1), 17551.
- Barry, P.H., Diamond, J.M., 1984. Effects of unstirred layers on membrane phenomena. *Physiol. Rev.* 61, 763–872.
- Bataille, A., Rousset, J., Marret, E., Bonnet, F., 2014. Ultrasonographic evaluation of gastric content during labour under epidural analgesia: a prospective cohort study. *Br. J. Anaesth.* 112 (4), 703–707.
- Beig, A., Fine-Shamir, N., Lindley, D., Miller, J.M., Dahan, A., 2017b. Advantageous solubility-permeability interplay when using amorphous solid dispersion (ASD) formulation for the BCS class IV P-gp substrate rifaximin: simultaneous increase of both the solubility and the permeability. *AAPS J.* 19 (3), 806–813.
- Beig, A., Miller, J.M., Lindley, D., Dahan, A., 2017a. Striking the optimal solubility–permeability balance in oral formulation development for lipophilic drugs: maximizing carbamazepine blood levels. *Mol. Pharm.* 14 (1), 319–327.
- Bergström, C., Holm, R., Jørgensen, S.A., Andersson, S.B.E., Artursson, P., Beato, S., Borde, A., Box, K., Brewster, M., Dressman, J., Feng, K.I., Halbert, G., Kostewicz, E., McAllister, M., Muenster, U., Thinnis, J., Taylor, R., Mullertz, A., 2014. Early pharmaceutical profiling to predict oral drug absorption: current status and unmet needs. *Eur. J. Pharm. Sci.* 57, 173–199. <https://doi.org/10.1016/j.ejps.2013.10.015>.
- Bermejo, M., Avdeef, A., Ruiz, A., Nalda, R., Ruell, J.A., Tsinman, O., González, I., Fernández, C., Sánchez, G., Garrigues, T.M., Merino, V., 2004. PAMPA—a drug absorption in vitro model 7. Comparing rat in situ, Caco-2, and PAMPA permeability of fluoroquinolones. *Eur. J. Pharm. Sci.* 21 (4), 429–441.
- Birchenough, G., Johansson, M.E., Gustafsson, J.K., Bergström, J.H., Hansson, G.C., 2015. New developments in goblet cell mucus secretion and function. *Mucosal Immunol.* 8 (4), 712–719. <https://doi.org/10.1038/mi.2015.32>.
- Blackburn, N.A., Johnson, I.T., 1983. The influence of guar gum on the movements of inulin, glucose and fluid in rat intestine during perfusion in vivo. *Pflügers Arch.* 397 (2), 144–148.
- Borgstrom B, Dahlqvist A, Lundh G, Sjoval J, B, B, Dahlqvist, A., Lundh, G., Sjoval, J., 1957. Studies of intestinal digestion and absorption in the human. *J. Clin. Invest.* 36, 1521–1536. doi:<https://doi.org/10.1172/JCI103549>.
- Borkenstein, R.F., 1971. Breath sampling and analyzing apparatus. U.S. Patent 3,552,930.
- Borkenstein, R.F., Smith, H.W., 1961. The breathalyzer and its applications. *Med. Sci. Law* 2 (1), 13–22.
- Braden, B., 2009. Methods and functions: breath tests. *Strking Pract. Res. Clin. Gastroenterol.* 23 (3), 337–352.
- Brouwers, J., Ingels, F., Tack, J., Augustijns, P., 2005. Determination of intraluminal theophylline concentrations after oral intake of an immediate- and a slow-release dosage form. *J. Pharm. Pharmacol.* 57, 987–996. <https://doi.org/10.1211/0022357056631>.
- Brouwers, J., Tack, J., Augustijns, P., 2007. Parallel monitoring of plasma and intraluminal drug concentrations in man after oral administration of fosamprenavir in the fasted and fed state. *Pharm. Res.* 24, 1862–1869. <https://doi.org/10.1007/s11095-007-9307-3>.
- Bryan, A.J., Kaur, R., Robinson, G., Thomas, N.W., Wilson, C.G., 1980. Histological and physiological studies on the intestine of the rat exposed to solutions of Myrj 52 and PEG 2000. *Int. J. Pharm.* 7 (2), 145–156.
- Cardim, N., Dalen, H., Voigt, J.U., Ionescu, A., Price, S., Neskovic, A.N., Edvardsen, T., Galderisi, M., Sicari, R., Donal, E. and Stefanidis, A., 2018. The use of handheld ultrasound devices: a position statement of the European Association of Cardiovascular Imaging (2018 update). *European Heart Journal-Cardiovascular Imaging*. <https://doi.org/https://doi.org/10.1093/ehjci/jej145>.
- Carrière, F., Grandval, P., Renou, C., Palomba, A., Priéri, F., Giallo, J., Henniges, F., Sander-Struckmeier, S., Laugier, R., 2005. Quantitative study of digestive enzyme secretion and gastrointestinal lipolysis in chronic pancreatitis. *Clin. Gastroenterol. Hepatol.* 3, 28–38.
- Carrière, F., Renou, C., Lopez, V., De Caro, J., Ferrato, F., Lengsfeld, H., De Caro, A., Laugier, R., Verger, R., 2000. The specific activities of human digestive lipases measured from the in vivo and in vitro lipolysis of test meals. *Gastroenterology* 119, 949–960.
- Carrière, F., Renou, C., Ransac, S., Lopez, V., De Caro, J., Ferrato, F., De Caro, A., Fleury, A., Sanwald-Ducray, P., Lengsfeld, H., Beglinger, C., Hadvary, P., Verger, R., Laugier, R., 2001. Inhibition of gastrointestinal lipolysis by orlistat during digestion of test meals in healthy volunteers. *Am. J. Physiol. Liver Physiol.* 281, G16–G28. <https://doi.org/10.1152/ajpgi.2001.281.1.G16>.
- Chaddock, G., Lam, C., Hoad, C.L., Costigan, C., Cox, E.F., Placidi, E., Thexton, I., Wright, J., Blackshaw, P.E., Perkins, A.C., Marciari, L., Gowland, P.A., Spiller, R.C., 2014. Novel MRI tests of orocecal transit time and whole gut transit time: studies in normal subjects. *Neurogastroenterol. Motil.* 26, 205–214. <https://doi.org/10.1111/nmo.12249>.
- Chang, F.Y., Lu, C.L., Chen, C.Y., Lee, S.D., Tsai, D.S., Fu, S.E., 2001. Applied potential tomography in liquid gastric emptying measurement: design, assembling, calibration, and clinical application. *Dig. Dis. Sci.* 46 (9), 1839–1845.
- Chen, D., Zhao, C.M., Lindström, E., Håkanson, R., 1999. Rat stomach ECL cells up-date of biology and physiology. In: *General Pharmacology: The Vascular System*. vol. 32(4). Pergamon, pp. 413–422. [https://doi.org/10.1016/S0306-3623\(98\)00221-3](https://doi.org/10.1016/S0306-3623(98)00221-3).
- Cheng, H., 1974. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine II. Mucous cells. *American Journal of Anatomy* 141 (4), 481–501. <https://doi.org/10.1002/aja.1001410404>.
- Chiloiro, M., Darconza, G., Piccioli, E., De Carne, M., Clemente, C., Riezzo, G., 2001. Gastric emptying and orocecal transit time in pregnancy. *J. Gastroenterol.* 36 (8), 538–543.
- Chu, S., Schubert, M.L., 2013. Gastric secretion. *Curr. Opin. Gastroenterol.* 29 (6), 636–641. <https://doi.org/10.1097/MOG.0b013e328365efc7>.
- Clarysse, S., Tack, J., Lammert, F., Duchateau, G., Reppas, C., Augustijns, P., 2009. Postprandial evolution in composition and characteristics of human duodenal fluids in different nutritional states. *J. Pharm. Sci.* 98, 1177–1192.
- Clear, N.J., Milton, A., Humphrey, M., Henry, B.T., Wulff, M., Nichols, D.J., Anziano, R.J., Wilding, I., 2001. Evaluation of the InteliSite capsule to deliver theophylline and frusemide tablets to the small intestine and colon. *Eur. J. Pharm. Sci.* 13, 375–384. [https://doi.org/10.1016/S0928-0987\(01\)00134-8](https://doi.org/10.1016/S0928-0987(01)00134-8).
- Clevers, H., 2013. Leading Edge the Intestinal Crypt, a Prototype Stem Cell Compartment. <https://doi.org/10.1016/j.cell.2013.07.004>.
- Cole, E.T., Scott, R.A., Cade, D., Connor, A.L., Wilding, I.R., 2004. In vitro and in vivo pharmacoscintigraphic evaluation of ibuprofen hypromellose and gelatin capsules. *Pharm. Res.* 21, 793–798. <https://doi.org/10.1023/B:PHAM.0000026430.73789.e6>.
- Creamer, B., Shorter, R.G., Bamforth, J., 1961. The turnover and shedding of epithelial cells part I the turnover in the gastro-intestinal tract. *Gut* 2, 110. <https://doi.org/10.1136/gut.2.2.110>.
- Dahan, A., Beig, A., Lindley, D., Miller, J.M., 2016. The solubility–permeability interplay and oral drug formulation design: two heads are better than one. *Adv. Drug Deliv. Rev.* 101, 99–107.
- Dainty, J., House, C.R., 1966. ‘Unstirred layers’ in frog skin. *J. Physiol.* 182 (1), 66–78.
- Darwich, A.S., Margolske, A., Pepin, X., Aarons, L., Galetin, A., Rostami-Hodjegan, A., Carlert, S., Hammarberg, M., Hilgendorf, C., Johansson, P., Karlsson, E., Murphy, D., Tannergren, C., Thörn, H., Yasin, M., Mazuir, F., Nicolas, O., Ramusovic, S., Xu, C., Pathak, S.M., Korjamo, T., Laru, J., Malkki, J., Pappinen, S., Tuunainen, J., Dressman, J., Hansmann, S., Kostewicz, E., He, H., Heimbach, T., Wu, F., Hofst, C., Pang, Y., Bolger, M.B., Huehni, E., Lukacova, V., Mullin, J.M., Szeto, K.X., Costales, C., Lin, J., McAllister, M., Modi, S., Rotter, C., Varma, M., Wong, M., Mitra, A., Bevernage, J., Biewenga, J., Van Peer, A., Lloyd, R., Shardlow, C., Langguth, P., Mishenzon, I., Nguyen, M.A., Brown, J., Lennernäs, H., Abrahamsson, B., 2016. IMI – Oral biopharmaceutics tools project – evaluation of bottom-up PBPK prediction success part 3: identifying gaps in system parameters by analysing in silico performance across different compound classes. *Eur. J. Pharm. Sci.* <https://doi.org/https://doi.org/10.1016/j.ejps.2016.09.037>.
- Darwiche, G., Almer, L.O., Björgell, O., Cederholm, C., Nilsson, P., 1999. Measurement of gastric emptying by standardized real-time ultrasonography in healthy subjects and diabetic patients. *J. Ultrasound Med.* 18 (10), 673–682.
- DeSesso, J.M., Jacobson, C.F., 2001. Anatomical and physiological parameters affecting gastrointestinal absorption in humans and rats. *Food Chem. Toxicol.* 39 (3), 209–228.
- di Cagno, M.P., Clarelli, F., Våbø, J., Lesley, C., Rahman, S.D., Cauzzo, J., Franceschini, E., Reardon, N., Stein, P.C., 2018. Experimental determination of drug diffusion coefficients in unstirred aqueous environments by temporally resolved concentration measurements. *Mol. Pharm.* 15 (4), 1488–1494.
- Diakidou, A., Vertzoni, M., Goumas, K., Söderlund, E., Abrahamsson, B., Dressman, J., Reppas, C., 2009. Characterization of the contents of ascending colon to which drugs are exposed after oral administration to healthy adults. *Pharm. Res.* 26, 2141–2151. <https://doi.org/10.1007/s11095-009-9927-x>.
- Diamond, J.M., 1966. A rapid method for determining voltage-concentration relations across membranes. *J. Physiol.* 183, 83–100.
- Diprose, W., Verster, F., Schauer, C., 2017. Re-examining physical findings with point-of-care ultrasound: a narrative review. *The New Zealand medical journal* 130 (1449), 46–51.
- Doll, W.J., Sandefer, E.P., Page, R.C., Ryo, U.Y., Friend, D.R., Azarnoff, D.L., Digenis, G.A., 1997. A bioavailability study of leuprolide acetate directly released in the ileum and colon of healthy human subjects using gamma scintigraphy and the InteliSite® capsule. *Pharm. Res.* 14, 654.
- Dressman, J.B., Berardi, R.R., Dermentzoglou, L.C., Russell, T.L., Schmaltz, S.P., Barnett, J.L., Jarvenpaa, K.M., 1990. Upper gastrointestinal (GI) pH in young, healthy men and women. *Pharm. Res.* 7, 756–761.
- Drozdziak, M., Gröer, C., Penski, J., Lapezjuk, J., Ostrowski, M., Lai, Y., Prasad, B., Unadkat, J.D., Siegmund, W., Oswald, S., 2014. Protein abundance of clinically relevant multidrug transporters along the entire length of the human intestine. *Mol. Pharm.* 11 (10), 3547–3555. <https://doi.org/10.1021/mp500330y>.
- Dubois, A., Eerdegwegh, P.V., Gardner, J.D., 1977. Gastric emptying and secretion in Zollinger-Ellison syndrome. *J. Clin. Invest.* 59, 255–263. <https://doi.org/10.1172/JCI108636>.
- Efentakis, M., Dressman, J.B., 1998. Gastric juice as a dissolution medium: surface tension and pH. *Eur. J. Drug Metab. Pharmacokin.* 23, 97–102.
- Erokhova, L., Horner, A., Ollinger, N., Silig, C., Pohl, P., 2016. The sodium glucose cotransporter SGLT1 is an extremely efficient facilitator of passive water transport. *J. Biol. Chem.* 291 (18), 9712–9720.
- European Medicines Agency, 2010. *Guideline on the Investigation of Bioequivalence*.
- Falavigna, M., Klitgaard, M., Brase, C., Ternullo, S., Škalko-Basnet, N., Flaten, G.E., 2018. Mucus-PVPA (mucus phospholipid vesicle-based permeation assay): an artificial permeability tool for drug screening and formulation development. *Int. J. Pharm.* 537

- (1–2), 213–222. <https://doi.org/10.1016/J.JPHARM.2017.12.038>.
- Faria, M., Pavin, E.J., Parisi, M.C.R., Lorena, S.L.S., Brunetto, S.Q., Ramos, C.D., Pavan, C.R., Mesquita, M.A., 2013. Delayed small intestinal transit in patients with long-standing type 1 diabetes mellitus: investigation of the relationships with clinical features, gastric emptying, psychological distress, and nutritional parameters. *Diabetes Technol. Ther.* 15 (1), 32–38.
- Ferrua, M.J., Singh, R.P., 2010. Modeling the fluid dynamics in a human stomach to gain insight of food digestion. *J. Food Sci.* 75, R151–R162.
- Ferrua, M.J., Xue, Z., Singh, R.P., 2014. On the kinematics and efficiency of advective mixing during gastric digestion – a numerical analysis. *J. Biomech.* 47, 3664–3673. <https://doi.org/10.1016/j.jbiomech.2014.09.033>.
- Food and Drug Administration, 2002. Guidance for Industry, Food-Effect Bioavailability and Fed Bioequivalence Studies.
- Georgaka, D., Butler, J., Kesioyglou, F., Reppas, C., Vertzoni, M., 2017. Evaluation of dissolution in the lower intestine and its impact on the absorption process of high dose low solubility drugs. *Mol. Pharm.* 14, 4181–4191.
- Gerardo, C.D., Cretu, E., Rohling, R., 2018. Fabrication and testing of polymer-based capacitive micromachined ultrasound transducers for medical imaging. *Microsystems & Nanoengineering* 4 (1), 19.
- Gleeson, J.P., Brayden, D.J., Ryan, S.M., 2017. Evaluation of PepT1 transport of food-derived antihypertensive peptides, Ile-Pro-Pro and Leu-Lys-Pro using in vitro, ex vivo and in vivo transport models. *Eur. J. Pharm. Biopharm.* 115, 276–284.
- Go, V.L., Hofmann, A.F., Summerskill, W.H., 1970. Simultaneous measurements of total pancreatic, biliary, and gastric outputs in man using a perfusion technique. *Gastroenterology* 58, 321–328.
- Green, K., Otori, T., 1970. Direct measurements of membrane unstirred layers. *J. Physiol.* 207, 93–102.
- Grimm, M., Ball, K., Scholz, E., Schneider, F., Sivert, A., Benamer, H., Kromrey, M.-L., Kühn, J.-P., Weitschies, W., 2019. Characterization of the gastrointestinal transit and disintegration behavior of floating and sinking acid-resistant capsules using a novel MRI labeling technique. *Eur. J. Pharm. Sci.* 129, 163–172. <https://doi.org/10.1016/j.ejps.2019.01.012>.
- Grimm, M., Koziolok, M., Kühn, J.-P., Weitschies, W., 2018a. Interindividual and intraindividual variability of fasted state gastric fluid volume and gastric emptying of water. *Eur. J. Pharm. Biopharm.* 127, 309–317. <https://doi.org/10.1016/j.ejpb.2018.03.002>.
- Grimm, M., Koziolok, M., Saleh, M., Schneider, F., Garbacz, G., Kühn, J.-P., Weitschies, W., 2018b. Gastric emptying and small bowel water content after administration of grapefruit juice compared to water and Isocaloric solutions of glucose and fructose: a four-way crossover MRI pilot study in healthy subjects. *Mol. Pharm.* 15, 548–559. <https://doi.org/10.1021/acs.molpharmaceut.7b00919>.
- Grimm, M., Scholz, E., Koziolok, M., Kühn, J.P., Weitschies, W., 2017. Gastric water emptying under fed state clinical trial conditions is as fast as under fasted conditions. *Mol. Pharm.* 14, 4262–4271. <https://doi.org/10.1021/acs.molpharmaceut.7b00623>.
- Gritti, I., Banfi, G., Roi, G.S., 2000. Pepsinogens: physiology, pharmacology pathophysiology and exercise. *Pharmacol. Res.* <https://doi.org/10.1006/phrs.1999.0586>.
- Hahn, T., Kozerke, S., Schwizer, W., Fried, M., Boesiger, P., Steingotter, A., 2011. Visualization and quantification of intestinal transit and motor function by real-time tracking of 19F labeled capsules in humans. *Magn. Reson. Med.* 66, 812–820. <https://doi.org/10.1002/mrm.22822>.
- Hamada, S.R., Garçon, P., Ronot, M., Kerever, S., Paugam-Burtz, C., Mantz, J., 2014. Ultrasound assessment of gastric volume in critically ill patients. *Intensive Care Med.* 40 (7), 965–972.
- Harris, M.S., Kennedy, J.G., Siegesmund, K.A., Yorde, D.E., 1988. Relationship between distention and absorption in rat intestine: I. effect of luminal volume on the morphology of the absorbing surface. *Gastroenterology* 94 (5), 1164–1171.
- Harwood, M.D., Achour, B., Russell, M.R., Carlson, G.L., Warhurst, G., Rostami-Hodjegan, A., 2015. Application of an LC–MS/MS method for the simultaneous quantification of human intestinal transporter proteins absolute abundance using a QconCAT technique. *J. Pharm. Biomed. Anal.* 110, 27–33.
- Haslam, I.S., O'Reilly, D.A., Sherlock, D.J., Kauser, A., Womack, C., Coleman, T., 2011. Pancreatoduodenectomy as a source of human small intestine for Ussing chamber investigations and comparative studies with rat tissue. *Biopharm. Drug Dispos.* 32, 210–221.
- Hauser, B., De Schepper, J., Cavelliers, V., Salvatore, S., Salvatoni, A., Vandenplas, Y., 2006. Variability of the 13C-acetate breath test for gastric emptying of liquids in healthy children. *J. Pediatr. Gastroenterol. Nutr.* 42 (4), 392–397.
- Hens, B., Brouwers, J., Corsetti, M., Augustijns, P., 2015. Gastrointestinal behavior of nano- and micro-sized fenofibrate: in vivo evaluation in man and in vitro simulation by assessment of the permeation potential. *Eur. J. Pharm. Sci.* 77, 40–47. <https://doi.org/10.1016/j.ejps.2015.05.023>.
- Hens, B., Brouwers, J., Corsetti, M., Augustijns, P., 2016a. Supersaturation and precipitation of posaconazole upon entry in the upper small intestine in humans. *J. Pharm. Sci.* 105, 2677–2684. <https://doi.org/10.1002/jps.24690>.
- Hens, B., Corsetti, M., Brouwers, J., Augustijns, P., 2016b. Gastrointestinal and systemic monitoring of posaconazole in humans after fasted and fed state administration of a solid dispersion. *J. Pharm. Sci.* 105, 2904–2912. <https://doi.org/10.1016/j.xphs.2016.03.027>.
- Hewitt, K.J., Agarwal, R., Morin, P.J., 2006. The claudin gene family: expression in normal and neoplastic tissues. *BMC Cancer. BioMed Central* 6, 186. <https://doi.org/10.1186/1471-2407-6-186>.
- Hidalgo, I.J., Hillgren, K.M., Grass, G.M., Borchardt, R.T., 1991. Characterization of the unstirred water layer in Caco-2 cell monolayers using a novel diffusion apparatus. *Pharm. Res.* 8 (2), 222–227.
- Higuchi, T., 1977. Prodrug, molecular structure and percutaneous delivery. In: Roche, E. (Ed.), *Design of Biopharm Properties through Prodrugs and Analogs*. American Pharmaceutical Association Academy of Pharmaceutical Sciences, Washington D.C., pp. 409–421.
- Hodges, L.A., Sime, K.A., Creech, L.A., Connolly, S.M., Barclay, S.T., Kwon, M.C., Jeon, B.J., Shim, S.M., Wang, H.S., Stevens, H.N.E., Park, J.S., 2013. Pharmacoscintigraphy confirms consistent tamsulosin release from a novel triple-layered tablet. *Int. J. Pharm.* 454, 41–45. <https://doi.org/10.1016/j.ijpharm.2013.06.065>.
- Huckaby, J.T., Lai, S.K., 2018. PEGylation for enhancing nanoparticle diffusion in mucus. In: *Advanced Drug Delivery Reviews*. vol. 124. Elsevier, pp. 125–139. <https://doi.org/10.1016/J.ADDR.2017.08.010>.
- Hunter, T.B., Taljanovic, M.S., 2003. Foreign bodies. *RadioGraphics* 23, 731–757. <https://doi.org/10.1148/rg.233025137>.
- Jantravid, E., Janssen, N., Reppas, C., Dressman, J.B., 2008. Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update. *Pharm. Res.* 25, 1663.
- Jenkins, D.J., Wolever, T.M., Leeds, A.R., Gassull, M.A., Haisman, P., Dilawari, J., Goff, D.V., Metz, G.L., Alberti, K.G., 1978. Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. *Br. Med. J.* 1 (6124), 1392–1394.
- Jéquier, E., Constant, F., 2010. Water as an essential nutrient: the physiological basis of hydration. *Eur. J. Clin. Nutr.* 64, 115–123. <https://doi.org/10.1038/ejcn.2009.111>.
- Johansson, M.E., Phillipson, M., Petersson, J., Velcich, A., Holm, L., Hansson, G.C., 2008. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc. Natl. Acad. Sci.* 105 (39), 15064–15069.
- Johansson, M.E.V., Larsson, J.M.H., Hansson, G.C., 2011. The two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host-microbial interactions. *Proc. Natl. Acad. Sci.* <https://doi.org/10.1073/pnas.1006451107>.
- Johnson, I.T., Gee, J.M., 1981. Effect of gel-forming gums on the intestinal unstirred layer and sugar transport in vitro. *Gut* 22 (5), 398–403.
- Johnson, I.T., Gee, J.M., 1982. Influence of viscous incubation media on the resistance to diffusion of the intestinal unstirred water layer in vitro. *Pflugers Arch.* 393 (2), 139–143.
- Johnston, N., Dettmar, P.W., Bishwokarma, B., Lively, M.O., Koufman, J.A., 2007. Activity/stability of human pepsin: implications for reflux attributed laryngeal disease. *Laryngoscope* 117, 1036–1039. <https://doi.org/10.1097/MLG.0b013e31804154c3>.
- Jones, B.E., Basit, A.W., Tuleu, C., 2012. The disintegration behaviour of capsules in fed subjects: a comparison of hypromellose (carrageenan) capsules and standard gelatin capsules. *Int. J. Pharm.* 424, 40–43. <https://doi.org/10.1016/j.ijpharm.2011.12.034>.
- Jordan, N., Newton, J., Pearson, J., Allen, A., 1998. A novel method for the visualization of the in situ mucus layer in rat and man. *Clin. Sci. (Lond)* 95, 97–106.
- Kagan, L., Lapidot, N., Afargan, M., Kirmayer, D., Moor, E., Mardor, Y., Friedman, M., Hoffman, A., 2006. Gastroretentive accordion pill: enhancement of riboflavin bioavailability in humans. *J. Control. Release* 113, 208–215. <https://doi.org/10.1016/j.jconrel.2006.03.022>.
- Kalantar-Zadeh, K., Berean, K.J., Ha, N., Chrimes, A.F., Xu, K., Grando, D., Ou, J.Z., Pillai, N., Campbell, J.L., Brkjača, R., Taylor, K.M., 2018. A human pilot trial of ingestible electronic capsules capable of sensing different gases in the gut. *Nature Electronics* 1 (1), 79.
- Kalantzi, L., Goumas, K., Kalioras, V., Abrahamsson, B., Dressman, J.B., Reppas, C., 2006. Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies. *Pharm. Res.* 23 (1), 165–176.
- Kararli, T.T., Searle, G.D., 1995. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals, biopharmaceutics & drug disposition. *Biopharm Drug Disposition* 16, 351–380.
- Kataoka, M., Sugano, K., da Costa Mathews, C., Wong, J.W., Jones, K.L., Masaoka, Y., Sakuma, S., Yamashita, S., 2012. Application of dissolution/permeation system for evaluation of formulation effect on oral absorption of poorly water-soluble drugs in drug development. *Pharm. Res.* 29, 1485–1494.
- Kenyon, C.J., Brown, F., McClelland, G.R., Wilding, I.R., 1998. The use of pharmacoscintigraphy to elucidate food effects observed with a novel protease inhibitor (saquinavir). *Pharm. Res.* 15, 417–422. <https://doi.org/https://doi.org/10.1023/a:1011972230829>.
- Kenyon, C.J., Nardi, R.V., Wong, D., Hooper, G., Wilding, I.R., Friend, D.R., 1997. Colonic delivery of dexamethasone: a pharmacoscintigraphic evaluation. *Aliment. Pharmacol. Ther.* 11, 205–213.
- Kerss, S., Allen, A., Garner, A., 1982. A simple method for measuring thickness of the mucus gel layer adherent to rat, frog and human gastric mucosa: influence of feeding, prostaglandin, N-acetylcysteine and other agents. *Clinical science (London, England: 1979)* 63 (2), 187–195. <https://doi.org/10.1042/CS0630187>. Portland Press Limited.
- Kidder, G.W., Cerejido, M., Curran, P.F., 1964. Transient changes in electrical potential differences across frog skin. *Am. J. Phys.* 207, 935–940.
- Kim, Y.S., Ho, S.B., 2010. Intestinal goblet cells and mucins in health and disease: recent insights and progress. *Current gastroenterology reports* 12 (5), 319–330.
- Kirch, J., Schneider, A., Abou, B., Hopf, A., Schaefer, U.F., Schneider, M., Schall, C., Wagner, C., Lehr, C.M., 2012. Optical tweezers reveal relationship between microstructure and nanoparticle penetration of pulmonary mucus. *Proc. Natl. Acad. Sci. U. S. A.* 109 (45), 18355–18360. <https://doi.org/10.1073/pnas.1214066109>.
- Klausner, E.A., Lavy, E., Friedman, M., Hoffman, A., 2003. Expandable gastroretentive dosage forms. *J. Control. Release* 90, 143–162. [https://doi.org/10.1016/S0168-3659\(03\)00203-7](https://doi.org/10.1016/S0168-3659(03)00203-7).
- Knutson, L., Odland, B., Hillgren, R., 1989. New technique for segmental jejunal perfusion in Man. *Am. J. Gastroenterol.* 84, 1278–1284. <https://doi.org/10.1111/j.1572-0241.1989.tb06168.x>.
- Koenigsnecht, M.J., Baker, J.R., Wen, B., Frances, A., Zhang, H., Yu, A., Zhao, T., Tsune, Y., Pai, M.P., Bleske, B.E., Zhang, X., Lionberger, R., Lee, A., Amidon, G.L., Hasler, W.L., Sun, D., 2017. In vivo dissolution and systemic absorption of immediate release

- ibuprofen in human gastrointestinal tract under fed and fasted conditions. *Mol. Pharm.* 14, 4295–4304. <https://doi.org/10.1021/acs.molpharmaceut.7b00425>.
- Kono, Y., Iwasaki, A., Matsuoka, K., Fujita, T., 2016. Effect of mechanical agitation on cationic liposome transport across an unstirred water layer in Caco-2 cells. *Biol. Pharm. Bull.* 39 (8), 1293–1299.
- Kourentas, A., Vertzoni, M., Stavrinoudakis, N., Symillides, A., Brouwers, J., Augustijns, P., Reppas, C., Symillides, M., 2016. An in vitro biorelevant gastrointestinal transfer (BioGIT) system for forecasting concentrations in the fasted upper small intestine: design, implementation, and evaluation. *Eur. J. Pharm. Sci.* 82, 106–114.
- Koziolek, M., Garbacz, G., Neumann, M., Weitschies, W., 2013. Simulating the post-prandial stomach: physiological considerations for dissolution and release testing. *Mol. Pharm.* 10, 1610–1622. <https://doi.org/10.1021/mp300604u>.
- Koziolek, M., Grimm, M., Becker, D., Iordanov, V., Zou, H., Shimizu, J., Wanke, C., Garbacz, G., Weitschies, W., 2014a. Investigation of pH and temperature profiles in the GI tract of fasted human subjects using the Intellicap(*) system. *J. Pharm. Sci.* 1–9. <https://doi.org/10.1002/jps.24274>.
- Koziolek, M., Grimm, M., Garbacz, G., Kühn, J.-P.P.J.P., Weitschies, W., Kühn, J.-P.P.J.P., Weitschies, W., 2014b. Intra-gastric volume changes after intake of a high-caloric, high-fat standard breakfast in healthy human subjects investigated by MRI. *Mol. Pharm.* 11, 1632–1639. <https://doi.org/10.1021/mp500022u>.
- Koziolek, M., Grimm, M., Schneider, F., Jedamzik, P., Sager, M., Kühn, J.P., Siegmund, W., Weitschies, W., 2016. Navigating the human gastrointestinal tract for oral drug delivery: uncharted waters and new frontiers. *Adv. Drug Deliv. Rev.* 101, 75–88. <https://doi.org/10.1016/j.addr.2016.03.009>.
- Koziolek, M., Schneider, F., Grimm, M., Modeß, C., Seekamp, A., Roustom, T., Siegmund, W., Weitschies, W., 2015. Intra-gastric pH and pressure profiles after intake of the high-caloric, high-fat meal as used for food effect studies. *J. Control. Release* 220, Part, 71–78. <https://doi.org/http://dx.doi.org/https://doi.org/10.1016/j.jconrel.2015.10.022>.
- Krug, S.M., Schulzke, J.D., Fromm, M., 2014. Tight junction, selective permeability, and related diseases. *Semin. Cell Dev. Biol.* 36, 166–176. <https://doi.org/10.1016/j.semcdb.2014.09.002>. Academic Press.
- Kvetina, J., Tachecí, I., Pavlík, M., Kopicová, M., Rejchrt, S., Douda, T., Kunes, M., Bures, J., 2015. Use of electrogastrography in preclinical studies of cholinergic and anticholinergic agents in experimental pigs. *Physiol. Res.* 64, S647–S652.
- Lai, S.K., O'Hanlon, D.E., Harrold, S., Man, S.T., Wang, Y.Y., Cone, R., Hanes, J., 2007. Rapid transport of large polymeric nanoparticles in fresh undiluted human mucus. *Proc. Natl. Acad. Sci. U. S. A.* 104 (5), 1482–1487.
- Lambert, G.P., Chang, R.T., Xia, T., Summers, R.W., Gisolfi, C.V., 1997. Absorption from different intestinal segments during exercise. *J. Appl. Physiol.* 83, 204–212. <https://doi.org/10.1152/jappp.1997.83.1.204>.
- Latorre, R., Sternini, C., De Giorgio, R., Greenwood-Van Meerveld, B., 2016. Enteroneuroendocrine cells: a review of their role in brain-gut communication. *Neurogastroenterology & Motility* 28 (5), 620–630. <https://doi.org/10.1111/nmo.12754>.
- Lee, J.S., 1983. Effects of stretching and stirring on water and glucose absorption by canine mucosal membrane. *J. Physiol.* 335 (1), 335–341.
- Lee, W.S., Davidson, G.P., Moore, D.J., Butler, R.N., 2000. Analysis of the breath hydrogen test for carbohydrate malabsorption: validation of a pocket-sized breath test analyser. *J. Paediatr. Child Health* 36 (4), 340–342.
- Lentle, R.G., De Loubens, C., 2015. A review of mixing and propulsion of chyme in the small intestine: fresh insights from new methods. *J. Comp. Physiol. B.* 185 (4), 369–387.
- Levitt, M.D., Strocchi, A., Levitt, D.G., 1992. Human jejunal unstirred layer: evidence for extremely efficient luminal stirring. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 262 (3), G593–G596.
- Lewis, L.D., Fordtran, J.S., 1975. Effect of perfusion rate on absorption, surface area, unstirred water layer thickness, permeability, and intraluminal pressure in the rat ileum in vivo. *Gastroenterology* 68 (6), 1509–1516.
- Li, M., de Graaf, I.A., van de Steeg, E., de Jager, M.H., Groothuis, G.M., 2016. The consequence of regional gradients of P-gp and CYP3A4 for drug-drug interactions by P-gp inhibitors and the P-gp/CYP3A4 interplay in the human intestine ex vivo. *Toxicol. in Vitro* 40, 26–33. <https://doi.org/10.1016/j.tiv.2016.12.002>.
- Lindahl, A., Ungell, A.L., Knutson, L., Lennernäs, H., 1997. Characterization of fluids from the stomach and proximal jejunum in men and women. *Pharm. Res.* 14, 497–502. <https://doi.org/10.1023/A:1012107801889>.
- Litou, C., Vertzoni, M., Goumas, C., Vasdekis, V., Xu, W., Kesiosoglou, F., Reppas, C., 2016. Characteristics of the human upper gastrointestinal contents in the fasted state under hypo- and A-chlorhydric gastric conditions under conditions of typical drug – drug interaction studies. *Pharm. Res.* 33, 1399–1412. <https://doi.org/10.1007/s11095-016-1882-8>.
- Loeffler, M., Birke, A., Winton, D., Potten, C., 1993. Somatic mutation, monoclonality and stochastic models of stem cell organization in the intestinal crypt. *J. Theor. Biol.* 160 (4), 471–491. <https://doi.org/10.1006/JTBI.1993.1031>.
- Longstreth, G.F., Malagelada, J.R., Go, V.L., 1975. The gastric response to a transpyloric duodenal tube. *Gut* 16, 777–780. <https://doi.org/10.1136/gut.16.10.777>.
- Lozoya-Agullo, I., Gonzalez-Alvarez, L., Zur, M., Fine-Shamir, N., Cohen, Y., Markovic, M., Garrigues, T.M., Dahan, A., Gonzalez-Alvarez, M., Merino-Sanjuán, M., Bermejo, M., 2018. Closed-loop doluisio (colon, small intestine) and single-pass intestinal perfusion (colon, jejunum) in rat—biophysical model and predictions based on Caco-2. *Pharm. Res.* 35 (1), 2.
- Lu, P.-J., Hsu, P.-I., Chen, C.-H., Hsiao, M., Chang, W.-C., Tseng, H.-H., Lin, K.-H., Chuah, S.-K., Chen, H.-C., 2010. Gastric juice acidity in upper gastrointestinal diseases. *World J. Gastroenterol.* 16, 5496–5501. <https://doi.org/10.3748/WJG.V16.I43.5496>.
- Lu, Z., Ding, L., Lu, Q., Chen, Yan-Hua, 2013. Tissue Barriers Claudins in intestines. In: Claudins in intestines, Tissue Barriers. vol. 1. pp. 3. <https://doi.org/10.4161/tisb.24978>.
- Lucas, M., 1983. Determination of acid surface pH in vivo in rat proximal jejunum. *Gut* 24 (8), 734–739.
- Lucas, M.L., Schneider, W., Haberich, F.J., Blair, J.A., 1975. Direct measurement by pH-microelectrode of the pH microclimate in rat proximal jejunum. *Proc. R. Soc. Lond. B* 192 (1106), 39–48.
- Mailman, D.A.V.I.D., Womack, W.A., Kviety, P.R., Granger, D.N., 1990. Villous motility and unstirred water layers in canine intestine. *Am. J. Physiol. Gastrointest. Liver Physiol.* 258 (2), G238–G246.
- Malagelada, J.R., Longstreth, G.F., Summerskill, W.H., Go, V.L., 1976. Measurement of gastric functions during digestion of ordinary solid meals in man. *Gastroenterology* 70, 203–210. [https://doi.org/https://doi.org/10.1016/S0016-5085\(76\)80010-8](https://doi.org/https://doi.org/10.1016/S0016-5085(76)80010-8).
- Marciani, L., Cox, E.F., Hoad, C.L., Pritchard, S., Totman, J.J., Foley, S., Mistry, A., Evans, S., Gowland, P.A., Spiller, R.C., 2010. Postprandial changes in small bowel water content in healthy subjects and patients with irritable bowel syndrome. *Gastroenterology* 138, 469–77, 477 e1. [https://doi.org/S0016-5085\(09\)01954-4](https://doi.org/S0016-5085(09)01954-4) [pii]10.1053/j.gastro.2009.10.055.
- Marciani, L., Gowland, P.A., Spiller, R.C., Manoj, P., Moore, R.J., Young, P., Al-Sahab, S., Bush, D., Wright, J., Fillery-Travis, A.J., 2000. Gastric response to increased meal viscosity assessed by echo-planar magnetic resonance imaging in humans. *J. Nutr.* 130, 122–127. <https://doi.org/10.1093/jn/130.1.122>.
- Marciani, L., Gowland, P.A., Spiller, R.C., Manoj, P., Moore, R.J., Young, P., Fillery-Travis, A.J., 2001. Effect of meal viscosity and nutrients on satiety, intragastric dilution, and emptying assessed by MRI. *Am. J. Physiol. Gastrointest. Liver Physiol.* 280, G1227–G1233.
- Markopoulos, C., Andreas, C., Vertzoni, M., Dressman, J., Reppas, C., 2015a. In-vitro simulation of luminal conditions for evaluation of performance of oral drug products: choosing the appropriate test media. *Eur. J. Pharm. Biopharm.* 93, 173–182.
- Markopoulos, C., Vertzoni, M., Symillides, M., Kesiosoglou, F., Reppas, C., 2015b. Two-stage single-compartment models to evaluate dissolution in the lower intestine. *J. Pharm. Sci.* 104, 2986–2997.
- Martin, N.E., Collison, K.R., Martin, L.L., Tardif, S., Wilding, I., Wray, H., Barrett, J.S., 2003. Pharmacoscintigraphic assessment of the regional drug absorption of the dual angiotensin-converting enzyme/neutral endopeptidase inhibitor, M100240, in healthy volunteers. *J. Clin. Pharmacol.* 43, 529–538. <https://doi.org/10.1177/0091270003252508>.
- Meredith, R.F., Torgue, J., Azure, M.T., Shen, S., Saddekni, S., Banaga, E., Carlisle, R., Bunch, P., Yoder, D., Alvarez, R., 2014. Pharmacokinetics and imaging of 212 Pb-TCMC-trastuzumab after intraperitoneal administration in ovarian cancer patients. *Cancer Biother. Radiopharm.* 29, 12–17. <https://doi.org/10.1089/cbr.2013.1531>.
- Misaka, S., Müller, F., Fromm, M.F., 2013. Clinical relevance of drug efflux pumps in the gut. *Curr. Opin. Pharmacol.* 13 (6), 847–852. <https://doi.org/10.1016/J.COPH.2013.08.010>. Elsevier.
- Mithani, S.D., Bakatselou, V., TenHoor, C.N., Dressman, J.B., 1996. Estimation of the increase in solubility of drugs as a function of bile salt concentration. *Pharm. Res.* 13, 163–167.
- Mudie, D.M., Murray, K., Hoad, C.L., Pritchard, S.E., Garnett, M.C., Amidon, G.L., Gowland, P.A., Spiller, R.C., Amidon, G.E., Marciani, L., 2014. Quantification of gastrointestinal liquid volumes and distribution following a 240 mL dose of water in the fasted state. *Mol. Pharm.* 11, 3039–3047. <https://doi.org/10.1021/mp500210c>.
- Murray, K., Hoad, C.L., Mudie, D.M., Wright, J., Heissam, K., Abrehart, N., Pritchard, S.E., Al Atwah, S., Gowland, P.A., Garnett, M.C., Amidon, G.E., Spiller, R.C., Amidon, G.L., Marciani, L., 2017. Magnetic resonance imaging quantification of fasted state colonic liquid pockets in healthy humans. *Mol. Pharm.* 14, 2629–2638. <https://doi.org/10.1021/acs.molpharmaceut.7b00095>.
- Murray, K., Wilkinson-Smith, V., Hoad, C., Costigan, C., Cox, E., Lam, C., Marciani, L., Gowland, P., Spiller, R.C., 2014. Differential effects of FODMAPs (fermentable oligo-, di-, mono-saccharides and polyols) on small and large intestinal contents in healthy subjects shown by MRI. *Am. J. Gastroenterol.* 109, 110–119. <https://doi.org/10.1038/ajg.2013.386>.
- Nakamura, K., Hirayama-Kurogi, M., Ito, S., Kuno, T., Yoneyama, T., Obuchi, W., Terasaki, T., Ohtsuki, S., 2016. Large-scale multiplex absolute protein quantification of drug-metabolizing enzymes and transporters in human intestine, liver, and kidney microsites by SWATH-MS: comparison with MRM/MS and HR-MRM/MS. *Proteomics* 16 (15–16), 2106–2117.
- Näslund, E., Bogefors, J., Grybäck, P., Jacobsson, H., Hellström, P.M., 2000. Gastric emptying: comparison of scintigraphic, polyethylene glycol dilution, and paracetamol tracer assessment techniques. *Scand. J. Gastroenterol.* 35, 375–379.
- Newcomer, A.D., McGill, D.B., Thomas, P.J., Hofmann, A.F., 1975. Prospective comparison of indirect methods for detecting lactase deficiency. *N. Engl. J. Med.* 293, 1232–1236.
- Newton, J.L., Jordan, N., Oliver, L., Strugala, V., Pearson, J., James, O.F., Allen, A., 1998. *Helicobacter pylori* in vivo causes structural changes in the adherent gastric mucus layer but barrier thickness is not compromised. *Gut* 43, 470–475.
- Newton, J.L., Jordan, N., Pearson, J., Williams, G.V., Allen, A., James, O.F.W., 2000. The adherent gastric antral and duodenal mucus gel layer thins with advancing age in subjects infected with *Helicobacter pylori*. *Gerontology* 46, 153–157. <https://doi.org/10.1159/000022151>.
- O'Connor, A., O'Moráin, C., 2014. Digestive function of the stomach. *Dig. Dis.* 32, 186–191. <https://doi.org/10.1159/000357848>.
- O'Dwyer, Patrick, J., Litou, C., Chara, Box, Karl J., Dressman, Jennifer B., Kostewicz, Edmund S., Kuentz, Martin, Reppas, Christos, 2019. In vitro methods to assess drug precipitation in the fasted small intestine – a PEARRL review. *J. Pharm. Pharmacol.* 71 (4), 536–556.
- Olivares-Morales, A., Lennernäs, H., Aarons, L., Rostami-Hodjegan, A., 2015. Translating

- human effective jejunal intestinal permeability to surface-dependent intrinsic permeability: a pragmatic method for a more mechanistic prediction of regional oral drug absorption. *AAPS J.* 1–16.
- Olsson, B., Wagner, Z.G., Mansson, P., Ragnarsson, G., 1995. A gamma scintigraphic study of the absorption of morphine from controlled-release tablets. *Int. J. Pharm.* 119, 223–229. [https://doi.org/10.1016/0378-5173\(94\)00400-Y](https://doi.org/10.1016/0378-5173(94)00400-Y).
- Parker, H.L., Liu, D., Curcic, J., Ebert, M.O., Schwizer, W., Fried, M., Steingoehter, A., 2017. Gastric and postgastric processing of 13C markers renders the 13C breath test an inappropriate measurement method for the gastric emptying of lipid emulsions in healthy adults. *J. Nutr.* 147 (7), 1258–1266.
- Parrott, N., Lave, T., 2010. Computer models for predicting drug absorption. In: *Oral Drug Absorption*. Wiley.
- Parrott, N., Lukacova, V., Fraczkiewicz, G., Bolger, M., 2009. Predicting pharmacokinetics of drugs using physiologically based modeling - application to food effects. *AAPS J.* 11, 45.
- Patel, N., Polak, S., Jamei, M., Rostami-Hodjegan, A., Turner, D., 2014. Quantitative prediction of formulation-specific food effects and their population variability from in vitro data with the physiologically-based ADAM model: a case study using the BCS/BDDCS class II drug nifedipine. *Eur. J. Pharm. Sci.* 57, 240–249.
- Paulick, A., Siegmund, W., 2013. Enterale Absorption von Paracetamol, Talinolol Und Amoxicillin Nach Oraler Gabe Mit 240 ml Wasser Und 240 ml Saccharose-Lösung. Inst. für Pharmakologie. University of Greifswald, Greifswald.
- Pedersen, B.L., Müllertz, A., Brøndsted, H., Kristensen, H.G., 2000. A comparison of the solubility of danazol in human and simulated gastrointestinal fluids. *Pharm. Res.* 17, 891–894.
- Pedersen, P.B., Vilmann, P., Bar-Shalom, D., Müllertz, A., Baldursdóttir, S., 2013. Characterization of fasted human gastric fluid for relevant rheological parameters and gastric lipase activities. *Eur. J. Pharm. Biopharm.* 85, 958–965. <https://doi.org/10.1016/j.ejpb.2013.05.007>.
- Pentafraqa, C., Symillides, M., McAllister, M., Dressman, J., Vertzoni, M., Reppas, C., 2019. The impact of food intake on the luminal environment and performance of oral drug products with a view to in vitro and in silico simulations: a PEARL review. *J. Pharm. Pharmacol.* 71 (4), 557–580.
- Péronnet, F., Mignault, D., du Souich, P., Vergne, S., Le Bellego, L., Jimenez, L., Rabasa-Lhoret, R., 2012. Pharmacokinetic analysis of absorption, distribution and disappearance of ingested water labeled with D2O in humans. *Eur. J. Appl. Physiol.* 112, 2213–2222. <https://doi.org/10.1007/s00421-011-2194-7>.
- Peters, S.A., et al., 2016. Predicting drug extraction in the human gut wall: assessing contributions from drug metabolizing enzymes and transporter proteins using pre-clinical models. *Clin. Pharmacokinet.* 55 (6), 673–696. <https://doi.org/10.1007/s40262-015-0351-6>. Springer International Publishing.
- Petrakis, O., Vertzoni, M., Angelou, A., Kesisoglou, F., Bentz, K., Goumas, K., Reppas, C., 2015. Identification of key factors affecting the oral absorption of salts of lipophilic weak acids: a case example. *J. Pharm. Pharmacol.* 67, 56–67. <https://doi.org/10.1111/jphp.12320>.
- Petri, N., Tannergren, C., Holst, B., Mellon, F.A., Bao, Y., Plumb, G.W., Bacon, J., O'Leary, K.A., Kroon, P.A., Knutson, L., Forsell, P., Eriksson, T., Lennernas, H., Williamson, G., 2003. Absorption/metabolism of sulforaphane and quercetin, and regulation of phase II enzymes, in human jejunum in vivo. *Drug Metab. Dispos.* 31, 805–813.
- Phillips, M., Cataneo, R.N., Cummin, A.R., Gagliardi, A.J., Gleeson, K., Greenberg, J., Maxfield, R.A., Rom, W.N., 2003. Detection of lung cancer with volatile markers in the breath. *Chest* 123 (6), 2115–2123.
- Pithavala, Y.K., Parr, A.F., Heizer, W.D., O'Connor-Semmes, R.L., Brouwer, K.L., 1998. Use of an externally activated drug delivery system to compare ranitidine absorption from various sites within the intestinal tract in humans. *Pharm. Res.* 15, 1869–1875.
- Poscente, M.D., Mintchev, M.P., 2017. Enhanced electrogastrography: a realistic way to salvage a promise that was never kept? *World J. Gastroenterol.* 23 (25), 4517–4528.
- Press, A.G., Hauptmann, I.A., Hauptmann, L., Fuchs, B., Fuchs, M., Ewe, K., Ramadori, G., 1998. Gastrointestinal pH profiles in patients with inflammatory bowel disease. *Aliment. Pharmacol. Ther.* 12, 673–678. <https://doi.org/10.1046/J.1365-2036.1998.00358.X>.
- Radwan, A., Wagner, M., Amidon, G.L., Langguth, P., 2014. Bio-predictive tablet disintegration: effect of water diffusivity, fluid flow, food composition and test conditions. *Eur. J. Pharm. Sci.* 57, 273–279. <https://doi.org/10.1016/j.ejps.2013.08.038>.
- Rahner, C., Mitic, L.L., Anderson, J.M., 2001. Heterogeneity in expression and subcellular localization of claudins 2, 3, 4, and 5 in the rat liver, pancreas, and gut. *Gastroenterology* 120 (2), 411–422. <https://doi.org/10.1053/GAST.2001.21736>. W. B. Saunders.
- Rana, S., Bhansali, A., Bhadada, S., Sharma, S., Kaur, J., Singh, K., 2011. Orocecal transit time and small intestinal bacterial overgrowth in type 2 diabetes patients from North India. *Diabetes Technol. Ther.* 13 (11), 1115–1120.
- Rana, S.V., Kochhar, R., Pal, R., Nagi, B., Singh, K., 2008. Orocecal transit time in patients in the chronic phase of corrosive injury. *Dig. Dis. Sci.* 53, 1797–1800.
- Rana, S.V., Malik, A., 2014. Hydrogen breath tests in gastrointestinal diseases. *Indian J. Clin. Biochem.* 29 (4), 398–405.
- Read, N.W., Barber, D.C., Levin, R.J., Holdsworth, C.D., 1977. Unstirred layer and kinetics of electrogenic glucose absorption in the human jejunum in situ. *Gut* 18 (11), 865–876.
- Read, N.W., Janabi, M.N. Al, Bates, T.E., Barber, D.C., 1983. Effect of gastrointestinal intubation on the passage of a solid meal through the stomach and small intestine in humans. *Gastroenterology* 84, 1568–1572. <https://doi.org/10.5555/URI:PII:0016508583903827>.
- Rees, W.D., Go, V.L., Malagelada, J.R., 1979. Simultaneous measurement of antroduodenal motility, gastric emptying, and duodenogastric reflux in man. *Gut* 20, 963–970. <https://doi.org/10.1136/gut.20.11.963>.
- Renou, C., Carrière, F., Ville, E., Grandval, P., Joubert-Collin, M., Laugier, R., 2001. Effects of lansoprazole on human gastric lipase secretion and intragastric lipolysis in healthy human volunteers. *Digestion* 63, 207–213. <https://doi.org/10.1159/000051891>.
- Reppas, C., Karatza, E., Goumas, C., Markopoulos, C., Vertzoni, M., 2015. Characterization of contents of distal ileum and cecum to which drugs/drug products are exposed during bioavailability/bioequivalence studies in healthy adults. *Pharm. Res.* 32, 3338–3349. <https://doi.org/10.1007/s11095-015-1710-6>.
- Riethorst, D., Mols, R., Duchateau, G., Tack, J., Brouwers, J., Augustijns, P., 2016. Characterization of human duodenal fluids in fasted and fed state conditions. *J. Pharm. Sci.* 105, 673–681. <https://doi.org/10.1002/jps.24603>.
- Ritz, P., Berrut, G., 2005. The importance of good hydration for the prevention of chronic diseases. *Nutr. Rev.* 63, S6–S13. <https://doi.org/https://doi.org/10.1301/nr.2005.jun.S6>.
- Rozehnal, V., Nakai, D., Hoepner, U., Fischer, T., Kamiyama, E., Takahashi, M., Yasuda, S., Mueller, J., 2012. Human small intestinal and colonic tissue mounted in the Ussing chamber as a tool for characterizing the intestinal absorption of drugs. *Eur. J. Pharm. Sci.* 46, 367–373.
- Sager, M., Jedamzik, P., Merdivan, S., Grimm, M., Schneider, F., Kromrey, M.-L., Hasan, M., Oswald, S., Kühn, J.-P., Koziolok, M., Weitschies, W., 2018. Low dose caffeine as a salivary tracer for the determination of gastric water emptying in fed and fasted state: a MRI validation study. *Eur. J. Pharm. Biopharm.* submitted, 443–452. <https://doi.org/https://doi.org/10.1016/j.ejpb.2018.03.011>.
- Saleem, A., Yap, J., Osman, S., Brady, F., Suttle, B., Lucas, S. V., Jones, T., Price, P.M., Aboagye, E.O., 2000. Modulation of fluorouracil tissue pharmacokinetics by eniluracil: in-vivo imaging of drug action. *Lancet* 355, 2125–2131. [https://doi.org/10.1016/S0140-6736\(00\)02380-1](https://doi.org/10.1016/S0140-6736(00)02380-1).
- Sandberg, T., Nilsson, M., Poulsen, J., Gram, M., Frøkjær, J., Østergaard, L., Drewes, A., 2015. A novel semi-automatic segmentation method for volumetric assessment of the colon based on magnetic resonance imaging. *Abdom. Imaging* 40, 2232–2241. <https://doi.org/10.1007/s00261-015-0475-z>.
- Sauter, M.M., Steingoehter, A., Curcic, J., Treier, R., Kuyumcu, S., Fried, M., Boesiger, P., Schwizer, W., Goetze, O., 2011. Quantification of meal induced gastric secretion and its effect on caloric emptying by magnetic resonance imaging (MRI). *Gastroenterology* 140, S-297. [https://doi.org/https://doi.org/10.1016/S0016-5085\(11\)61193-1](https://doi.org/https://doi.org/10.1016/S0016-5085(11)61193-1).
- Schiller, C., Fröhlich, C.P., Giessmann, T., Siegmund, W., Mönnikes, H., Hosten, N., Weitschies, W., 2005. Intestinal fluid volumes and transit of dosage forms as assessed by magnetic resonance imaging. *Aliment. Pharmacol. Ther.* 22, 971–979. <https://doi.org/APT2683> [pii] 10.1111/j.1365-2036.2005.02683.x.
- Schneider, F., Grimm, M., Koziolok, M., Modef, C., Dokter, A., Roustom, T., Siegmund, W., Weitschies, W., 2016. Resolving the physiological conditions in bioavailability and bioequivalence studies: comparison of fasted and fed state. *Eur. J. Pharm. Biopharm.* 108, 214–219. <https://doi.org/10.1016/j.ejpb.2016.09.009>.
- Schubert, M.L., Peura, D.A., 2008. Control of gastric acid secretion in health and disease. *Reviews in Basic and Clinical Gastroenterology* 134, 1842–1860. <https://doi.org/10.1053/j.gastro.2008.05.021>.
- Sebrechts, T., Perlas, A., Abbas, S., Van de Putte, P., 2018. Serial gastric ultrasound to evaluate gastric emptying after Prokinetic therapy with Domperidone and erythromycin in a surgical patient with a full stomach: a case report. *A&A Practice* 11 (4), 106–108.
- Sellers, R.S., Morton, D., 2014. The Colon: from banal to brilliant. *Toxicol. Pathol.* 42 (1), 67–81. <https://doi.org/10.1177/0192623313505930>. 2014 Jan.
- Shi, X., Passe, D.H., 2010. Water and solute absorption from carbohydrate- electrolyte solutions in the human proximal small intestine: a review and statistical analysis. *Int J Sport Nutr Exerc Metab* 20, 427–442.
- Shibayama, T., Morales, M., Zhang, X., Martínez-Guerrero, L.J., Berteloot, A., Secomb, T.W., Wright, S.H., 2015. Unstirred water layers and the kinetics of organic cation transport. *Pharm. Res.* 32 (9), 2937–2949.
- Siissalo, S. and T. Heikkinen, A. (2012) 'In vitro methods to study the interplay of drug metabolism and efflux in the intestine', *Curr. Drug Metab.*, 14(1), pp. 102–111. doi: <https://doi.org/10.2174/1389200211309010102>.
- Simonian, H.P., Vo, L., Doma, S., Fisher, R.S., Parkman, H.P., 2005. Regional postprandial differences in pH within the stomach and gastroesophageal junction. *Dig. Dis. Sci.* 50, 2276–2285. <https://doi.org/10.1007/s10620-005-3048-0>.
- Sjoberg, A., Lutz, M., Tannergren, C., Wingolf, C., Borde, A., Ungell, A.L., 2013. Comprehensive study on regional human intestinal permeability and prediction of fraction absorbed of drugs using the Ussing chamber technique. *Eur. J. Pharm. Sci.* 48, 166–180.
- Sjögren, E., Abrahamsson, B., Augustijns, P., Becker, D., Bolger, M.B.M.B., Brewster, M., Brouwers, J., Flanagan, T., Harwood, M., Heinen, C., Holm, R., Juretschke, H.-P.P.H.-P., Kubbinga, M., Lindahl, A., Lukacova, V., Münster, U., Neuhoof, S., Nguyen, M.A.M.A., Peer, A. van, Reppas, C., Hodjegan, A.R.A.R., Tannergren, C., Weitschies, W., Wilson, C.G., Zane, P., Lennernas, H., Langguth, P., van Peer, A., Reppas, C., Hodjegan, A.R.A.R., Tannergren, C., Weitschies, W., Wilson, C.G.C.G., Zane, P., Lennernas, H., Langguth, P., 2014. In vivo methods for drug absorption - comparative physiologies, model selection, correlations with in vitro methods (IVIVC), and applications for formulation/API/excipient characterization including food effects. *Eur. J. Pharm. Sci.* 57, 99–151. <https://doi.org/10.1016/j.ejps.2014.02.010>.
- Sjögren, Erik, Westergren, Jan, Grant, Iain, Hanisch, Gunilla, Lindfors, Lennart, Lennernas, Hans, Abrahamsson, Bertil, Tannergren, Christer, 2013. In silico predictions of gastrointestinal drug absorption in pharmaceutical product development: application of the mechanistic absorption model GI-Sim. *Eur. J. Pharm. Sci.* 49, 679–698.
- Smithson, K.W., Millar, D.B., Jacobs, L.R., Gray, G.M., 1981. Intestinal diffusion barrier: unstirred water layer or membrane surface mucous coat? *Science* 214 (4526), 1241–1244 11.

- Soybel, D.J., 2005. Anatomy and physiology of the stomach. *Surg. Clin. North Am.* 85, 875–894. <https://doi.org/10.1016/j.suc.2005.05.009>.
- Srivastava, A., Belemkar, S., Bonde, S.C., Nagpal, P., 2014. Pharmacoscintigraphy and its applications: a review. *Inven. Rapid Pharmacokinet. Pharmacodyn.* 1–8.
- Steed, K.P., Hooper, G., Monti, N., Strolin Benedetti, M., Fornasini, G., Wilding, I.R., 1997. The use of pharmacoscintigraphy to focus the development strategy for a novel 5-ASA colon targeting system ("TIME CLOCK" system). *J. Control. Release* 49, 115–122. [https://doi.org/10.1016/S0168-3659\(97\)00062-X](https://doi.org/10.1016/S0168-3659(97)00062-X).
- Steingotter, A., 2015. Magnetic resonance imaging for the assessment of gastrointestinal function and fat digestion. In: 4th International Conference on Food Digestion, (Naples).
- Steingotter, A., Weishaupt, D., Kunz, P., Mäder, K., Lengsfeld, H., Thumshirn, M., Boesiger, P., Fried, M., Schwizer, W., 2003. Magnetic resonance imaging for the in vivo evaluation of gastric-retentive tablets. *Pharm. Res.* 20, 2001–2007. <https://doi.org/10.1023/B:PHAM.0000008049.40370.5a>.
- Sternini, C., Anselmi, L., Rozengurt, E., 2008. Enteroendocrine cells: a site of "taste" in gastrointestinal chemosensing. Current opinion in endocrinology, diabetes, and obesity 15 (1), 73–78. <https://doi.org/10.1097/MED.0b013e3282f43a73>. NIH Public Access.
- Strugala, V., Dettmar, P.W., Pearson, J.P., 2008. Thickness and continuity of the adherent colonic mucus barrier in active and quiescent ulcerative colitis and Crohn's disease. *Int. J. Clin. Pract.* 62 (5), 762–769. <https://doi.org/10.1111/j.1742-1241.2007.01665.x>. Wiley/Blackwell (10.1111).
- Sümpelmann, A.E., Sümpelmann, R., Lorenz, M., Eberwien, I., Dennhardt, N., Boethig, D., Russo, S.G., 2017. Ultrasound assessment of gastric emptying after breakfast in healthy preschool children. *Pediatr. Anesth.* 27 (8), 816–820.
- Swidsinski, A., Loening-Baucke, V., Theissig, F., Engelhardt, H., Bengmark, S., Koch, S., Lochs, H., Dörffel, Y., 2007. Comparative study of the intestinal mucus barrier in normal and inflamed colon. *Gut* 56 (3), 343–350.
- Szentkuti, L., Lorenz, K., 1995. The thickness of the mucus layer in different segments of the rat intestine. *Histochem. J.* 27 (6), 466–472.
- Tannergren, C., Knutson, T., Knutson, L., Lennernäs, H., 2003. The effect of ketoconazole on the in vivo intestinal permeability of fexofenadine using a regional perfusion technique. *Br. J. Clin. Pharmacol.* 55, 182–190. <https://doi.org/10.1046/J.1365-2125.2003.01722.X>.
- Teitelbaum, E.N., Vaziri, K., Zettervall, S., Amdur, R.L., Orkin, B.A., 2013. Intraoperative small bowel length measurements and analysis of demographic predictors of increased length. *Clin. Anat.* 26 (7), 827–832. <https://doi.org/10.1002/ca.22238>.
- Teubl, B.J., Absenger, M., Fröhlich, E., Leitinger, G., Zimmer, A., Roblegg, E., 2013. The oral cavity as a biological barrier system: design of an advanced buccal in vitro permeability model. *Eur. J. Pharm. Biopharm.* 84 (2), 386–393. <https://doi.org/10.1016/J.EJPB.2012.10.021>.
- Thombre, A.G., Shamblin, S.L., Malhotra, B.K., Connor, A.L., Wilding, I.R., Caldwell, W.B., 2015. Pharmacoscintigraphy studies to assess the feasibility of a controlled release formulation of ziprasidone. *J. Control. Release* 213, 10–17. <https://doi.org/10.1016/j.jconrel.2015.06.032>.
- Thomson, A.B., Dietschy, J.M., 1980. Intestinal kinetic parameters: effects of unstirred layers and transport preparation. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 239 (5), G372–G377.
- Tsunashima, D., Kawamura, A., Murakami, M., Sawamoto, T., Undre, N., Brown, M., Groenewoud, A., Keirns, J.J., Holman, J., Connor, A., Wylde, H., Wilding, I., Ogawara, K.I., Sako, K., Higaki, K., First, R., 2014. Assessment of tacrolimus absorption from the human intestinal tract: open-label, randomized, 4-way crossover study. *Clin. Ther.* 36, 748–759. <https://doi.org/10.1016/j.clinthera.2014.02.021>.
- Tuleu, C., Basit, A.W., Waddington, W.A., Ell, P.J., Newton, J.M., 2002. Colonic delivery of 4-aminosalicylic acid using amylose-ethylcellulose-coated hydroxypropylmethylcellulose capsules. *Aliment. Pharmacol. Ther.* 16, 1771–1779. <https://doi.org/10.1046/j.1365-2036.2002.01327.x>.
- Van Den Abeele, J., Brouwers, J., Mattheus, R., Tack, J., Augustijns, P., 2016. Gastrointestinal behavior of weakly acidic BCS class II drugs in man - case study diclofenac potassium. *J. Pharm. Sci.* 105, 687–696. <https://doi.org/10.1002/jps.24647>.
- Van Den Abeele, J., Rubbens, J., Brouwers, J., Augustijns, P., 2017a. The dynamic gastric environment and its impact on drug and formulation behaviour. *Eur. J. Pharm. Sci.* 96, 207–231. <https://doi.org/10.1016/j.ejps.2016.08.060>.
- Van Den Abeele, J., Brouwers, J., Deloosse, E., Tack, J., Augustijns, P., 2017b. The effect of sparkling water on intraluminal formulation behavior and systemic drug performance. *J. Pharm. Sci.* 106, 2472–2482. <https://doi.org/10.1016/j.xphs.2017.03.039>.
- Van Den Abeele, J., Schilderink, R., Schneider, F., Mols, R., Minekus, M., Weitschies, W., Brouwers, J., Tack, J., Augustijns, P., 2017c. Gastrointestinal and systemic disposition of diclofenac under fasted and fed state conditions supporting the evaluation of in vitro predictive tools. *Mol. Pharm.* 14, 4220–4232. <https://doi.org/10.1021/acs.molpharmaceut.7b00253>.
- Van Eyken, P., et al., 2014. The Normal biopsy: Mucosa and submucosa. In: *Colitis*. Springer International Publishing, Cham, pp. 1–16. https://doi.org/10.1007/978-3-319-08028-4_1.
- Varum, F.J.O., Merchant, H.A., Basit, A.W., 2010. Oral modified-release formulations in motion: the relationship between gastrointestinal transit and drug absorption. *Int. J. Pharm.* 395, 26–36. <https://doi.org/10.1016/j.ijpharm.2010.04.046>.
- Varum, F.J.O., et al., 2012. Mucus thickness in the gastrointestinal tract of laboratory animals. *J. Pharm. Pharmacol.* 64 (2), 218–227. <https://doi.org/10.1111/j.2042-7158.2011.01399.x>. Wiley/Blackwell (10.1111).
- Vertzoni, M., Diakidou, A., Chatziliadis, M., Söderlind, E., Abrahamsson, B., Dressman, J.B., Reppas, C., 2010. Biorelevant media to simulate fluids in the ascending colon of humans and their usefulness in predicting intracolonic drug solubility. *Pharm. Res.* 27, 2187–2196. <https://doi.org/10.1007/s11095-010-0223-6>.
- Vertzoni, M., Markopoulos, C., Symillides, M., Goumas, C., Imanidis, G., Reppas, C., 2012. Luminal lipid phases after administration of a triglyceride solution of danazol in the fed state and their contribution to the flux of danazol across Caco-2 cell monolayers. *Mol. Pharm.* 9, 1189–1198.
- Ville, E., Carrière, F., Renou, C., Laugier, R., 2002. Physiological study of pH stability and sensitivity to pepsin of human gastric lipase. *Digestion* 65, 73–81. <https://doi.org/10.1159/000057708>.
- Walraven, J., Brouwers, J., Spriet, I., Tack, J., Annaert, P., Augustijns, P., 2011. Effect of pH and comedication on gastrointestinal absorption of posaconazole: monitoring of intraluminal and plasma drug concentrations. *Clin. Pharmacokinet.* 50, 725–734. <https://doi.org/10.2165/11592630-000000000-00000>.
- Weitschies, W., Wilson, C.G., 2011. In vivo imaging of drug delivery systems in the gastrointestinal tract. *Int. J. Pharm.* 417, 216–226. [https://doi.org/S0378-5173\(11\)00662-4](https://doi.org/S0378-5173(11)00662-4) [pii]. <https://doi.org/10.1016/j.ijpharm.2011.07.031>.
- Westergaard, H., Dietschy, J.M., 1974. Delineation of the dimensions and permeability characteristics of the two major diffusion barriers to passive mucosal uptake in the rabbit intestine. *J. Clin. Invest.* 54, 718–732.
- Wilding, I.R., Connor, A.L., Carpenter, P., Rordorf, C., Branson, J., Milosavljevic, S., Scott, G., 2004. Assessment of lumiracoxib bioavailability from targeted sites in the human intestine using remotely activated capsules and gamma scintigraphy. *Pharm. Res.* 21, 443–446. <https://doi.org/10.1023/B:PHAM.0000019297.07378.bd>.
- Wilding, I.R., Coupe, A.J., Davis, S.S., 2001. The role of γ -scintigraphy in oral drug delivery. *Adv. Drug Deliv. Rev.* 46, 103–124. [https://doi.org/10.1016/S0169-409X\(00\)0135-6](https://doi.org/10.1016/S0169-409X(00)0135-6).
- Wilding, I.R., Davis, S.S., Sparrow, R.A., Ziemniak, J.A., Heald, D.L., 1995. Pharmacoscintigraphic evaluation of a modified release (Geomatrix®) diltiazem formulation. *J. Control. Release* 33, 89–97. [https://doi.org/10.1016/0168-3659\(94\)00066-4](https://doi.org/10.1016/0168-3659(94)00066-4).
- Wilding, I.R., Hardy, J.G., Maccari, M., Ravelli, V., Davis, S.S., 1991. Scintigraphic and pharmacokinetic assessment of a multiparticulate sustained release formulation of diltiazem. *Int. J. Pharm.* 76, 133–143. [https://doi.org/10.1016/0378-5173\(91\)90351-N](https://doi.org/10.1016/0378-5173(91)90351-N).
- Williams, H.D., Sassene, P., Kleberg, K., Bakala-N'Goma, J.-C., Calderone, M., Jannin, V., Igonin, A., Partheil, A., Marchaud, D., Jule, E., Vertommen, J., Maio, M., Blundell, R., Benamer, H., Carrière, F., Müllertz, A., Porter, C.J.H., Pouton, C.W., 2012. Toward the establishment of standardized in vitro tests for lipid-based formulations, part 1: method parameterization and comparison of in vitro digestion profiles across a range of representative formulations. *J. Pharm. Sci.* 101, 3360–3380. <https://doi.org/10.1002/jps.23205>.
- Wilson, C.G., 2010. The transit of dosage forms through the colon. *Int. J. Pharm.* 395, 17–25. <https://doi.org/10.1016/j.ijpharm.2010.04.044>.
- Wilson, C.G., O'Mahony, B., Connolly, S.M., Cantarini, M.V., Farmer, M.R., Dickinson, P.A., Smith, R.P., Swaisland, H.C., 2009. Do gastrointestinal transit parameters influence the pharmacokinetics of gefitinib? *Int. J. Pharm.* 376, 7–12. <https://doi.org/10.1016/j.ijpharm.2009.04.008>.
- Wilson, C.G., Washington, N., Greaves, J.L., Kamali, F., Rees, J.A., Sempik, A.K., Lampard, J.F., 1989. Bimodal release of ibuprofen in a sustained-release formulation: a scintigraphic and pharmacokinetic open study in healthy volunteers under different conditions of food intake. *Int. J. Pharm.* 50, 155–161. [https://doi.org/10.1016/0378-5173\(89\)90140-3](https://doi.org/10.1016/0378-5173(89)90140-3).
- Winn, P.M., Smith, T.E., Campbell, A.D., Huf, E.G., 1964. Sodium diffusion in epidermis and corium of frog skin and in Ringer-agar gel. *J. Cell. Comp. Physiol.* 64 (3), 371–387.
- Winne, D., 1978. Dependence of intestinal absorption in vivo on the unstirred layer. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 304, 175–181.
- Wong, C.A., Loffredi, M., Ganchiff, J.N., Zhao, J., Wang, Z., Avram, M.J., 2002. Gastric emptying of water in term pregnancy. *Anesthesiology. The Journal of the American Society of Anesthesiologists* 96 (6), 1395–1400.
- Wyse, C.A., Yam, P.S., Sutton, D.G.M., Christley, R.M., Hotchkiss, J.W., Love, S., Preston, T., Mills, C.A., Glidle, A., Cumming, D.R.S., Cooper, J.M., 2004. Current and future uses of breath analysis as a diagnostic tool. *The Veterinary Record* 154 (12), 353.
- Yu, A., Baker, J.R., Fioritto, A.F., Wang, Y., Luo, R., Li, S., Wen, B., Bly, M., Tsume, Y., Koenigsnecht, M.J., Zhang, X., Lionberger, R., Amidon, G.L., Hasler, W.L., Sun, D., 2017. Measurement of in vivo gastrointestinal release and dissolution of three locally acting mesalamine formulations in regions of the human gastrointestinal tract. *Mol. Pharm.* 14, 345–358. <https://doi.org/10.1021/acs.molpharmaceut.6b00641>.
- Zhao, M., Chen, D., 2012. The ECL cell: relay station for gastric integrity. *Curr. Med. Chem.* 19 (1), 98–108. <https://doi.org/10.2174/092986712803414060>.